INTEGRATED ORGANIC MANAGEMENT OF CABBAGE APHID ON BRUSSELS SPROUTS

BY

ALINA SOPHIA HARRIS

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THESIS

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This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Agricultural Science by:

Thesis Director, Dr. Rebecca Grube Sideman,
Extension Professor of Sustainable Agriculture

Iago Hale, Associate Professor,
Agriculture, Nutrition, and Food Systems

Anna Wallingford,
Extension Integrated Pest Management Specialist

On July 18, 2019

Approval signatures are on file with the University of New Hampshire Graduate School.

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ABSTRACT

INTEGRATED ORGANIC MANAGEMENT

OF CABBAGE APHID

ON BRUSSELS SPROUTS

by

Alina Sophia Harris

University of New Hampshire

Growers across the globe and in Northeastern United States have reported economically damaging populations of cabbage aphid (*Brevicoryne brassicae*) in Brussels sprouts (*Brassicacea*). The pest affects the *Brassicacea* family, which includes economically important agronomic, horticultural, and forage crops. However, cabbage aphid management in certified organic systems is very challenging because tools are limited and reports evaluating efficacy of insecticides used in organic systems are sparse. There are natural insect predators and parasitoids of aphids that may serve as biological control agents whose populations can be augmented using insectary plants. Use of alyssum insectary intercropping has been successful for control of this pest in California but use of this practice is untested in the Northeast. Our research aimed to find an integrated approach to managing cabbage aphid on Brussels sprouts by using chemical and biological pest management strategies in conjunction. Our overall goal was to explore the relationship between Brussels sprout and cabbage aphid in organic agroecosystems, with three specific objectives.

Our first objective was to evaluate three organic insecticides for their efficacy in controlling cabbage aphid. We compared Azera (azadirachtin and pyrethrins), AzaGuard (azadirachtin), and M-pede (Potassium salts of fatty acids) against an untreated control. In 2016 a rotation of M-Pede and Azera provided moderate control of cabbage aphid with significantly more marketable clean (aphid-free) sprouts as compared to the unsprayed control. In 2017, Azera and AzaGuard treatments had significantly less aphids on 28 Sept 2017 than the control and M-Pede treatments, but by final harvest there were no significant differences between treatments. Aphid numbers continued to rise in all treatments into September 2017 until a spontaneous epizootic resulted in a collapse of aphid numbers. In both 2017 and 2018 two different entomopathogenic (insect-attacking) fungi were identified on cabbage aphid. Based on these observations, we conclude that Azera and AzaGuard insecticides may provide moderate control of cabbage aphid and further investigation is needed on years without fungal epizootics.

Our second objective was to evaluate seven species of insectary plants in the field for their ability to attract predators and parasitoids of cabbage aphids. Insectary plants were observed over ten sample dates (July through October) for hoverfly densities and sweep net samples were taken for hoverfly species identification. Alyssum, buckwheat, cilantro, and dill had greater hoverfly densities than calendula, phacelia, and fennel. Alyssum was found to be a low maintenance plant that hosts the most prevalent aphid-eating hoverfly species (*Toxomerus marginatus*) from July until frost.

Our third objective was to determine whether parasitism of the cabbage aphid varied with proximity to insectary plants. We used both sticky traps and visual observation on Brussels sprouts leaves to count aphids, predators, and parasites at distances ranging from four to 107 feet away. In 2017, we found that predation and parasitism rates were not significantly different at

distances ranging from four to 107 feet from the insectary plants. In 2018, aphid populations were not high enough to repeat the experiment. From 2017 results, we concluded that proximity of insectary plants from the Brussels sprout crop did not correlate with predation or parasitism in distances observed in our study.

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CHAPTER 1:

LITERATURE REVIEW

The *Brassicaceae* (*Brassica*) family is comprised of about 340 genera and 3,700 species. It is presumed to have been domesticated at around 1000 B.C.E. and has been cultivated for centuries across continents. The *Brassica* family which includes Brussels sprouts, broccoli, cauliflower, kale, kohlrabi, radish, mustard, canola and cabbage is of vast economic importance world-wide (Pedras and Yaya, 2010). According to the Food and Agricultural Organization, in 2016, there were over 100 million tons of cabbage and other *Brassicas* produced by 153 countries or territories in the world (FAO, 2016).

Growers across the globe have reported economically damaging populations of cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) (Bayhan et al., 2007; Ellis et al., 1996a). Common descriptors of cabbage aphid include "gray aphid", "winter aphid", or "mealy cabbage aphid" (Jankowska, 2005). Cabbage aphid is a cosmopolitan *Brassica* pest that severely affects agronomic crops (oil-seed brassicas), forage crops, and horticultural crops (vegetables and ornamentals) (Gabrys, 2008; Singh and Ellis, 1993). Economic losses include reduction in yield, storability, and marketability of the crop (Gadhave and Gange, 2016; Shah et al., 2004). Cabbage aphids have been reported to cause losses of up to 85% of crop yields and are vectors of about 20 plant viruses (Gabrys, 2008).

Infestations of cabbage aphid have been reported in California (Brennan, 2016; Bugg et al., 2008), West Indian islands (Alam, 1992), Serbia (Marcic et al., 2007), France (Neuville et al., 2016), Brazil (Bacci et al., 2009; Mussury and Fernandes, 2002), Yunnan province of China (Chen et al., 2007), Poland (Jankowska, 2005), Lithuania (Duchovskienė et al., 2012), the United

Kingdom (Shah et al., 2004), Ethiopia (Nahusenay and Abate, 2018), Kenya (Bahana and Karuhize, 1986), Iran (Amini et al., 2012) Lebanon, Syria, Turkey, Israel, Egypt, and Iraq (Bodenheimer and Swirski, 1957).

Cabbage aphid lifecycle

For integrated pest management to be effective it is paramount to understand the pest's lifecycle. Successful pest management requires the pest life cycle to be disrupted. However, disruption of the cabbage aphid is particularly difficult with its complex lifecycle that enables survival in harsh conditions through polymorphism. The lifecycle of the cabbage aphid is comprised of four nymphal instars. One cabbage aphid generation can develop in seven to 10 days, but time period may be effected by temperature and relative humidity (Gabrys, 2008). Each instar, or life stage, has its own strategic function in the infestation of the host plant and are described below (Figure 1).

(1) The first instar are females, described as **virginoparous aptera.** A "virginoparous" aphid indicates they were born from asexual reproduction and "apterous" means they will not form wings. Their bodies are grayish-green (1.6-2.6 mm long) with a dark head and are also covered in a gray-white mealy wax (Gabrys, 2008). These non-winged aphids are the predominant instar found throughout the growing season and give birth to live nymphs that immediately start feeding on the host plant. Newborn nymphs molt four times before reaching mature size. Each non-winged aphid produces about 30 to 50 nymphs during this instar (Hafez, 1961; Herrick, 1911; Hughes, 1963).

(2) Next, winged progeny are formed. These can fly to new host plants to start new colonies of aphids (Hughes, 1963). A combination of seasonal environmental changes (photoperiod), overcrowding, and host plant quality decline induce the non-winged aptera to

birth the second instar, called **alatae** ("winged aphids") (Gabrys, 2008). These aphids are also females and look similar to their mothers (1.6-2.8mm long with a black head) except they form wings and their outer coating of wax is thinner than that of non-winged aphids which make them appear more green (Gabrys, 2008). Winged aphids can travel up to 1 km (Chen et al., 2007) and their movement depends largely on wind currents (Hughes, 1963; Kennedy et al., 1959). Like other aphids, cabbage aphid cannot combat wind currents greater than 2ft/sec (60cm/sec) (Hughes, 1963). Thus, winged aphid dispersal from neighboring areas is largely dependent on prevailing wind. Upon landing on a host plant, one winged female can asexually produce a new colony of progeny rapidly (Bugg et al., 2008). Winged females are parthenogenic and viviparous; they reproduce asexually and give birth to fully formed non-winged aphids. They produce about 15 to 30 nymphs in this instar (Hafez, 1961; Herrick, 1911; Hughes, 1963). In parts of the world with mild winters the lifecycle is "anholocyclic" and the entire lifecycle is comprised of only the first two female instars that rely on asexual reproduction (Gabrys, 2008; Hafez, 1961). However, in different colder climates with harsher seasonal variation, cabbage aphids have adapted their lifecycle to become "holocyclic." This biologically strategic reproduction tactic survives the changes in weather by producing a third (sexual) and fourth (asexual) instar (Gabrys, 2008).

(3) The third instar is comprised of **apterous oviparae** (winged, egg-laying) females and **alatae** (non-winged) males. The female ovum is fertilized by the male sperm cell to produce fertilized eggs that are oviposited on *Brassica* host plants that can survive harsh temperatures and environmental changes (Hughes, 1963). This sexual phase of the holocyclic lifecycle is induced by changing environmental factors such as cold temperatures (below 10-15°C for at least 24 hours (Gabrys, 2008)), latitude (i.e. photoperiod), and potentially relative humidity

(Bodenheimer and Swirski, 1957; Hafez, 1961). In turn, different regions in the world report different findings. Low temperatures of 10°C in Israel (**30°**52'N latitude and 35°0'E longitude) did not induce sexual reproduction and the cabbage aphid overwintered as live asexual, non-winged adults (Bodenheimer and Swirski, 1957; Hafez, 1961). In contrast, in France (**46°**36'N latitude and 1°53'E longitude), higher temperatures of 20°C have been cited to induce sexual reproduction and produce some overwintering eggs, although the majority overwinter as non-winged adults (Bodenheimer and Swirski, 1957; Bonnemaison, 1951; Hafez, 1961). The present study takes place in Durham, NH (**43°**8'N latitude and 70°55'W longitude) and has a latitude that falls in between Israel and France. Researchers in Australia (**24°** 46'S latitude and 134°45'E longitude) and Egypt (**26°**15' latitude and 29°16'E longitude) found that cabbage aphid overwintered as asexual, non-winged aphids. In contrast, cabbage aphid in Finland (**63°**14'N latitude and 25°55'E longitude) have been found to reproduce sexually and exclusively overwinter as eggs (Hafez, 1961). Using the latitude of the places where sexual reproduction in cabbage aphid is reported, it appears that latitudes below 30° (North or South) result in asexual reproduction, and latitudes 46° (North) and above result in partial or exclusively sexual reproduction to survive through the winter.

(4) In the spring the eggs hatch into **fundatrix**, sometimes called "stem mothers" that congregate at the top portion of the host plant. Fundatrix are parthenogenic and give birth to the first nymphal instar of non-winged aphids (Hughes, 1963). The top portion of biennial plants such as Brussels sprouts, kale and wild relatives makes for an ideal concentration of food reserves for newly emerged cabbage aphids in the apical flower meristem region. This food

source is temporary for the cabbage aphids because the plant translocates its reserves to the seeds and in turn, the plant begins to senesce (Hughes, 1963).

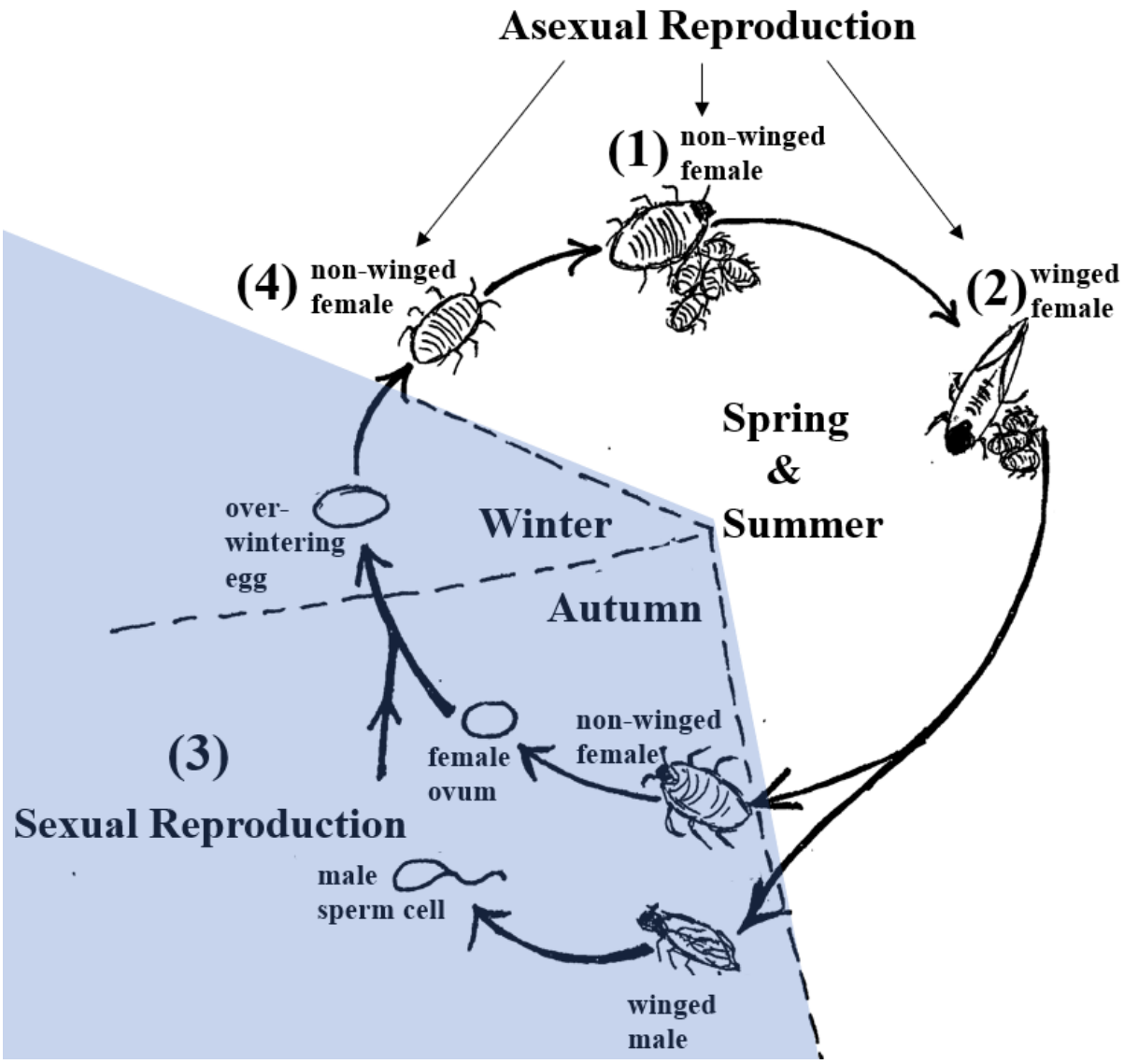


Figure 1. The lifecycle of cabbage aphid in a cold climate with all four life stages. Life stages (1), (2), and (4) use asexual reproduction whereas life stage (3) uses sexual reproduction and is shaded.

Brussels sprouts as a cabbage aphid host

Of the various *Brassica* species that are hosts to cabbage aphid, Brussels sprouts are particularly affected. The physiology of the Brussels sprout plant is particularly vulnerable to cabbage aphid infestations. Its large leaf canopy shelters insects on the main stem part of the plant. Cabbage aphids are apt to hide in crevices of leaves or under the leaf canopy (Figure 2A). Moreover, leaves are prone to curling or folding in response to cabbage aphid infestations on the leaf margin (Bahana and Karuhize, 1986; Lammerink, 1968; Seaman, 2016). Thus, aphids protected by leaves may remain untouched by insecticides that work by contact, making it difficult to obtain effective chemical control. Furthermore, Brussels sprouts are a long season crop that can range from about 93 to 110 days in maturity. As a long season crop, the Brussels sprouts are suitable hosts for the cabbage aphid, since winged aphids distribute themselves into agricultural crops starting in the beginning of July in New Hampshire (Sideman, Levy, and Harris; unpublished) and sometimes as early as June.

Figure 2. (A) View of Brussels sprouts underneath leaf canopy; (B) characteristic yellow on the opposite side of the leaf from a cabbage aphid infestation.

Cabbage aphid prefers new growth but is found throughout plant (low, middle, and high leaves) as well as on both sides of leaves (Rimaz and Valizadegan, 2013) and detailed scouting is needed to find the first aphids of the season. Once infested, plants may show yellowing on the opposite side of the leaf from aphid infestations (Figure 2B) or stunted growth (Bahana and Karuhize, 1986). Cabbage aphid is difficult to control, particularly in certified organic field systems with our current knowledge and tools.

Cabbage aphid in Northeastern United States

Trends in winter *Brassica* production in the Northeastern United States may play a part in the survival of cabbage aphid. Farmers that follow cultural practices of tilling-in crop residue may still grow *Brassica* crops in protected environments (i.e. high tunnels, low tunnels, row covers). Two *Brassica* crops, kale and mustards, are commonly grown during the winter in these environments. These protected or temperature-controlled environments provide mild temperatures, shelter, and host crops for cabbage aphid. Thus, these conditions may possibly allow for asexual phases to survive the winter. Hafez, (1961) found that continued low temperatures of 4.9°C and 5.5°C did not allow for survival of young nymphs or completion of maturity, whereas temperatures of 13.1°C and 17.8°C allowed for their development. Unheated protected environments in the Northeast reach temperatures below 5°C, however, these temperatures fluctuate from very cold to much warmer during the day. Further studies are needed to test the effects of protected environments on cabbage aphid survival.

With cold temperatures in the Northeast, we hypothesize aphids in the spring hatch from eggs on overwintered *Brassicas* or are birthed from live aphid adults that have survived in microclimates with less harsh temperatures. It is possible that these microclimates exist on crop residue below the soil surface (deeper than a few cm) or in protected environments. Preliminary

data has shown the first aphids found in crop fields are winged aphids and appear in random locations in July of most years (Sideman and Levy, unpublished). Once winged aphids land on a plant, they reproduce asexually and rapidly produce a large colony of non-winged nymphs. Once these colonies reach a certain density, new winged aphids are formed, and they spread from these original colonies (Hughes, 1963).

A recent study conducted at the University of New Hampshire Woodman Research Farm showed a decline in marketable yield of Brussels sprouts when cabbage aphid was not controlled properly in an organic system (Levy and Sideman, 2017; Levy and Sideman, unpublished). In a survey conducted in 2017 (Levy and Sideman, unpublished), commercial farmers reported crop losses caused by cabbage aphid ranging from 0 to 100%. Thirty-three farmers from Vermont, New Hampshire, Massachusetts, New York, Maine, and Rhode Island responded to the survey, growing between 6 and 14.4 acres of Brussels sprouts. Using grower-reported price, yield, and cabbage aphid loss data, the average Brussels sprout crop value per grower was between $7,000 and $25,000; the total potential crop value ranged from $197,000 to $713,000. Cabbage aphid infestations resulted in a range of losses. The range spanned from an average of $2,000 to $11,184 per grower, with a total estimated loss ranging from $54,000 to $300,000. The majority experienced 0% to 50% total crop loss attributed to cabbage aphid.

Cabbage aphid demands insecticide and labor expenses (Zhang and Hassan, 2003) that decrease profitability in Brussels sprouts (Bacci et al., 2009). Insecticides have historically and currently been one of the main tools used in attempt to control cabbage aphid around the world (Bacci et al., 2009; Bahana and Karuhize, 1986; Bodenheimer and Swirski, 1957; Ellis et al., 2000, 1996b; Zhang and Hassan, 2003) and locally in the Northeastern United States (Levy and Sideman, unpublished). Of the 33 attendees at a recent webinar focused on cabbage aphid

management in the Northeast, 88% described themselves as certified organic or preferred using insecticides that are approved for certified organic operations. This suggests that certified organic and low-input growers are having trouble managing this pest with the tools available to them (Scheufele, S, unpublished). Currently, reports evaluating the efficacy of organic-approved insecticides against cabbage aphid are sparse, and farmers are discouraged from growing Brussels sprouts (Levy and Sideman, unpublished).

Cabbage aphids are particularly difficult to control for several reasons. Their polymorphism of both asexual and sexual reproduction allows them to survive extreme abiotic conditions as well as produce winged forms to disperse to different conditions (Bacci et al., 2009; Bodenheimer and Swirski, 1957). Since aphid colonies reproduce rapidly, an ideal control agent should act quickly on the first winged aphids that appear in the crop field. Non-winged aphids stay mostly stationary on the leaf and have a piercing and sucking mouthpart (Shah et al., 2004) that feeds only on the phloem (sap) of their host plant (Rimaz and Valizadegan, 2013). Because of this specialized feeding mechanism, ingestion of the outer surface of the plant where foliar insecticides are applied is largely bypassed (Bodenheimer and Swirski, 1957). For this reason, aphid insecticide types must be either contact insecticides or systemic insecticides that make the phloem toxic (Bodenheimer and Swirski, 1957). Systemic pesticides are typically toxic to target organisms that are phytophagous (eat the plant), but not toxic towards beneficial predators and parasitoids (Bodenheimer and Swirski, 1957). Systemic pesticides may maintain their efficacy for longer than foliar spray insecticides that rapidly degrade in field environments (Henn and Weinzierl, 1989).

Bacci et al. (2009) reports failures of controlling cabbage aphid in Brazil with current insecticides and discusses the complexity of management with insecticides. *Brassica* crops also

have other insect pests, such as lepidopteran caterpillars and fleabeetles (Tukahirwa and Coaker, 1982) as well as fungal disease, such as *Alternaria* (Nowicki et al., 2012). In these complex, multi-faceted agroecosystems, there are many confounding variables that may help one aspect but may hinder another. For instance, a grower may apply an insecticide or fungicide in hopes of controlling a target pest, but it may have detrimental consequences for non-target beneficial organisms. Repeated use of broad-spectrum insecticides against pests have caused secondary outbreaks (Walter, 1999), insect resistance to insecticides (Rimaz and Valizadegan, 2013), and deleterious effects to beneficial non-target insects (Walter, 1999). Selective insecticides have modes of action that target a specific pest but can minimize harmful effects to natural enemies (Bacci et al., 2009; Giles and Obrycki, 1997). Bacci et al. (2009) predict that insecticides are likely to remain as one of the tools for the management of cabbage aphid but advocate for integrated pest management that also utilizes biological control. Use of practices that protect or minimize harm to beneficial organisms are forms of "conservation" biological control.

Conservation biological control

Biological control is the use of living organisms to reduce pest populations. "Classic" biological control and "inundative release" both introduce insect enemies into an area to control pests (Laubertie, 2007). However, this experiment mainly focuses on "conservation" biological control, which takes a systems approach to recognize the multi-faceted interactions between organisms in the agroecosystem (Bacci et al., 2009). Two forms of conservation biological control are investigated in the current study: (1) the use of insecticides against cabbage aphid that limit the harm of beneficial organisms such as predatory insects, parasitic insects, and entomopathogenic fungi; (2) manipulation of the agroecosystem to attract and enhance fitness of

antagonists of aphids already present in the region to increase their effectiveness as biological control agents (Bacci et al., 2009; Debach and Rosen, 1991).

Organic-approved insecticides

Commercialized products. Though other commercialized insecticides are available, this study focuses on insecticides that are permitted for use in certified organic systems and will be referred to as "organic-approved" insecticides. A local crop reference guide lists a plethora of organic-approved insecticides for treatment of aphids (McKeag and Dicklow, 2017). Seaman (2016) compiled a literature review of experiments that evaluated the efficacy of organic-approved insecticide materials on control of aphids. Active ingredients that were found to be efficacious against aphids in more than half of experiments reviewed by Seaman (2016) include **azadirachtin** (trade names: Aza-Direct, AzaGuard, Azera, AzaMaz, AzaSol, Azatrol-EC, Ecozin Plus 1.2 ME, Molt-X), **neem oil** (trade name: Trilogy), and **pyrethrins** (trade names: Azera, Pyganic EC 1.4 II, Pyganic EC 5.0 II). **Potassium salts of fatty acids** (M-pede) were found to have poor efficacy alone but acted as a synergist in insecticide mixtures. A number of other active ingredients were listed for aphid control, however, literature is lacking on the efficacy of the following active ingredients: **garlic juice** (tradenames: Biolink, BioLink insect and bird repellant, Envirepel 20, Garlic Barrier AG, BioRepel), **Rosemary oil+peppermint oil** (tradename: Ecotec), **cinnamon oil** (tradename: GrasRoots), **chromobacterium subtsuggae str. PRAAA4-1** (trade name: Grandevo), ***Isaria fumosorosea Apopka* str. 97,** formerly known as ***Paecilomyces fumosoroseus*** (tradename: PFR-97 20% WDG), ***Beauveria bassiana* strain GHA** (tradename: Mycotrol ESO), and ***Lecanicillium muscarium*,** previously known as ***Verticillium lecanii*** (trade name: Mycotal).

Of the above listed active ingredients, most are botanical insecticides that are naturally derived from plants (Henn and Weinzierl, 1989). Potassium salts of fatty acids are generally regarded as distinct from botanical insecticides, but are produced from fatty acids that come from either natural plant or animal sources (Henn and Weinzierl, 1989). Other active ingredients listed are biological pesticides with living organisms as the active ingredient. *Beauveria bassiana* strain GHA, *Lecanicillium muscarium*, and *Isaria fumosorosea* Apopka str. 97 are entomopathogenic fungi that parasitize aphids. *Chromobacterium subtsuggae* str. PRAAA4-1 is an insecticidal bacterium.

Uncommercialized products. There are botanical products that have not been commercialized that have potential for greenhouse or controlled environment use against cabbage aphid. Pavela (2006) demonstrated that essential oils of catnip (*Nepeta cataria*) and lavender (*Lavandula augustifola*) applied as fumigant aerosols caused 90% mortality in cabbage aphid in a greenhouse experiment.

Entomopathogenic fungi

Naturally-occurring entomopathogenic fungi in the environment. Insect pathogens play a role in insect population dynamics (Chen et al., 2007). It is not uncommon for spontaneous outbreaks of naturally occurring entomopathogenic fungi to cause epizootics in the field that collapse aphid populations (Chen et al., 2007). Entomophthorales is an order of entomopathogenic fungus that is parasitic to aphids in crop fields, especially in the autumn during periods of high humidity (Reyes-Rosas et al., 2012; Shah et al., 2004). In particular, *Pandora neoaphidis* (homotypic synonym *Erynia neoaphidis)* has been demonstrated to reduce cabbage aphid by 90% on canola in Mexico (Reyes-Rosas et al., 2012). With certain temperatures and relative humidity conditions in the field it is possible for many aphid

populations to plummet rapidly, however Shah et al. (2004) conclude that fungal attacks occur too late in the season to reliably control aphids. Though most of the aphids are killed during such outbreaks, there can be significant negative effects on marketability and storability of these crops due to presence of dead fungi-infected aphids and prior aphid feeding damage (Shah et al., 2004). Though fungal outbreaks may take place later in the year when aphid colonies are more established, it is likely that entomopathogenic fungi spores are present with the arrival of the first winged aphids into the field (Chen et al., 2007).

Spores of aphidophagous fungi are distributed through infected winged aphids (Chen et al., 2007). The spores of the fungi remain dormant on the body of the aphid until climatic conditions are suitable for germination, such as the high humidity caused by abundant rainfall (Chen et al., 2007). Chen et al. (2007) evaluated entomopathogenic fungi on trapped winged aphids in China (latitude N, 25°04; E, 102°41) from the top of a 6-story building (altitude 200 meters). Over an 11-month period Chen et al. (2007) trapped aphids and observed them for fungal parasitism in petri dishes on cabbage leaves (23 ± 2°C and 16L:8D photoperiod). They concluded that Entomophthorales spores were present on trapped aphids throughout the entire 11 months and was responsible for over 90% of aphid mortality. Higher cabbage aphid mortality due to Entomophthorales correlated with the higher relative humidity from May to August compared to the rest of the observation period. They also identified the species of fungi and found that 95% of cabbage aphid was infected by three dominant species (*P. neoaphidis*, *E. planconiana*, or *B. bassiana*). Aphids infected with *P. neoaphidis* died between one to four days post-trapping, whereas aphids infected by *B. bassiana* died between three to six days after trapping.

In a greenhouse setting, Hall and Burges (1979) found mixed results on the efficacy of *Lecanicillium muscarium* (previously known as *Verticillium lecanii* (Zimmermann)), as a control agent on aphids. They found that a single spray of a solution that included *L. muscarium* spores controlled green peach aphid for the remainder of the crop production, whereas its efficacy against chrysanthemum aphid (*Macrosiphoniella sanborni*) and *Brachycaudus helichrysi* was variable and plant quality was not satisfactory.

Seven wild isolates of *P. neoaphidis* were evaluated for their pathogenicity against cabbage aphid in a laboratory setting (Reyes-Rosas et al., 2012). The fungal isolates were collected in Mexico from three types of aphids (bird cherry-oat aphid (*Rhopalosiphum padi* (L.)), corn aphid (*Rhopalosiphum maidis* (Fitch)), and the green peach aphid). Three of these isolates had over a 70% mortality rate on cabbage aphid, suggesting that some isolates of *P. neoaphidis* are a potential biological control agent of cabbage aphid (Reyes-Rosas et al., 2012). They have been shown to be fatal within a 24 hour period in laboratory conditions (24 ± 1 °C, 65 ± 10% RH) (Kim et al., 2013; Vu et al., 2007).

Effects of insecticides on entomopathogenic fungi. From a practical standpoint, it is important to remember that some synthetic pesticides have been found to be incompatible with entomopathogenic fungi (Neves et al., 2001; Sajjad Ali et al., 2018). Some insecticides used for the control of aphids have fungicidal properties, such as neem products (Girish and Shankara, 2008; Henn and Weinzierl, 1989). It is plausible that insecticide applications aimed to control cabbage aphid could prevent or minimize the beneficial effects of naturally occurring entomopathogenic fungi. In the same way, it is plausible that *Brassicas* crops sprayed with fungicides aimed to control fungus that attacks the host plant (i.e. *Alternaria*) may hurt or prevent naturally occurring entomopathogenic fungi. Studies that evaluated the efficacy of

mixtures that combined entomopathogenic fungi (*B. bassiana*) with neem products report mixed results. Sajjad Ali et al, 2018 reports that a binary mixture of neem extract and *B. bassiana* were less effective in their control of wheat aphid (*Sitobion avenae*) than neem extract or *B. bassiana* on their own. They hypothesized the lower mortality rate and higher fecundity rates attributed to the binary mixture may be due to neem leaf extract causing deleterious effects on mycelial growth, conidiogenesis, and spore germination of *B. bassiana* when neem extract concentration of 5% or higher was used (Castiglioni et al., 2003).

In contrast, laboratory studies have demonstrated binary mixtures of entomopathogenic fungi and botanical insecticides to be compatible or even more efficacious than when used singly (Mohan et al., 2007; Russo et al., 2015). A laboratory study showed that neem can have synergistic effects with specific strains of *B. bassiana* (Mohan et al., 2007). Mohan et al, 2007 tested 30 isolates of *B. bassiana* for compatibility with azadirachtin of which 23 were compatible and even showed synergist insecticidal effects on a lepidopteran pest. A binary mixture of *B. bassiana* and eucalyptus (*Eucalyptus camaldulensis*) extract resulted in higher mortality rates of wheat aphid than only *B. bassiana* and eucalyptus used on their own (Sajjad Ali et al., 2018). Like neem, eucalyptus extract contains a terpenoid compound that is a feeding deterrent (decreases appetite) and growth regulator (of maturation and reproduction) (Russo et al., 2015). Most insecticides do not specify compatibility with entomopathogenic fungi; further research is needed to understand these complexities and their implications for commercial field crops.

Abiotic parameters or agricultural practices that may affect cabbage aphid

Effects of temperature on aphids. Several experiments have studied the effects of abiotic parameters on aphids. Temperature can affect which type of cabbage aphid instar is produced (sexual or asexual reproduction), rate of development (Hafez, 1961), and birthing rate

(how many aphids are produced in a time period) (Bonnemaison, 1951). Hafez, (1961) found that low temperatures of 4.9°C and 5.5°C did not allow for survival of young nymphs or completion of maturity. Cabbage aphid developed at temperatures as low as 13.1°C but optimum development occurred at 28.2°C. Temperatures of 30.9°C resulted in survival of only a few individuals that developed more slowly. Days to development of cabbage aphid ranged from eight days with optimal temperatures to 43 days with non-optimal temperatures. Bonnemaison (1951) evaluated cabbage aphid birthing rate on cabbage leaf discs at constant temperatures of 17°C and 24°C, as well as temperatures that alternated between 17°C and 24°C and found the fluctuating temperature treatment to have the highest birthing rate. A growth chamber study that evaluated aphids on lettuce demonstrated that lower temperatures increased aphid rate of reproduction (Bugg et al., 2008), whereas field observations from Hughes (1963) state that colder weather seemed to decrease the reproductive rate of cabbage aphid.

Effects of nitrogen fertility on aphids. Petitt et al. (1994) demonstrated that differing nitrogen levels provided to cucumber plants affected reproduction of *Aphis gossypii* and also on pepper plants with green peach aphid. Tariq et al. (2013) found that higher nitrogen concentrations in Brussels sprout leaves were positively correlated with cabbage aphid fecundity (birthing rate). Van Emden (1965) found similar results; cabbage aphid fecundity was highest on aging leaves which they attributed to higher concentrations of nitrogen in the leaf phloem. Gabrys, (2008) reported that high levels of nitrogen applied to the soil of field crops positively affected aphid population development.

Effects of drought stress on aphids. Researchers report dry years to have particularly damaging infestations, compared to seasons with regular rain (Bahana and Karuhize, 1986; Petherbridge and Mellor, 1936). Petherbridge and Mellor (1936) cited particularly intense aphid

infestations during the hot dry season of 1921. Hafez (1961) cited similar fluctuations of cabbage aphid, likely due to weather. They had substantial numbers of cabbage aphid in the hot dry year of 1959 in two different field locations, whereas the number of cabbage aphids in 1960 with regular rain were less than a quarter of the year prior. In England, Pollard (1969) also cited a season with virtually no cabbage aphids on single plants and attributed it to regular rain (7.06 inches or 179.3mm in about a one-month period). However, the mechanism for this phenomenon is not clear. Possibilities include that host plant water status may affect aphid populations, aphids may be physically knocked off of plants by rain, or increased humidity may favor entomopathogenic fungi. Broadbent (1953) who studied aphids of potatoes and Dunn and Wright (1955) who studied pea aphids (*Acyrthosiphon pisum* (Harris)) attributed declines in summer aphid populations to physical knockoff from heavy rain.

Greenhouse studies found mixed results with water stress by hand-watering plants at the base. Morris (1992) used the aphid *Aphis varians* and the host plant fireweed (*Epilobium angustifolium*). They found aphid colonies decline in size when plants were watered one time per four days compared with treatments that were watered daily, or every other day (Morris, 1992). However, Khan et al. (2010) demonstrated that cabbage aphid was not affected by drought stress; cabbage aphid populations sizes did not change between water-stressed plants and their non-water stressed counterparts.

Tariq et al. (2013) evaluated Brussels sprout plants that had undergone moderate and severe levels of drought stress compared to a regular watering regime and demonstrated that plants that had undergone moderate levels of drought stress increased the performance and number of cabbage aphid. Plants with moderate to high levels of drought stress also had higher nitrogen concentrations that were positively correlated with cabbage aphid fecundity (Tariq et

al., 2013). In the same study, they also measured glucosinolate concentration, (plant defense compounds produced by many *Brassica* crops) and found that when root herbivory and moderate drought were combined, there was a 62% increase in the concentration of glucosinolate.

Genetic resistance to cabbage aphid. There have been at least 39 field and laboratory experiments evaluating *Brassica* genotypes for resistance to cabbage aphid (Singh and Ellis, 1993). In these studies, 950 *Brassica* genotypes were tested, 93 of which had moderate to high levels of genetic resistance to cabbage aphid. Two forms of genetic resistance were found. **Antixenosis** affects the behavior of an insect pest and usually is expressed as the pest showing a non-preference to a resistant plant compared with a susceptible (not resistant) plant. **Antibiosis** often results in increased mortality or reduced longevity and reproduction of the pest. In *Brassicas*, antixenosis resistance to cabbage aphid is associated with red and glossy leaf phenotypes, whereas antibiosis does not have clear phenotypic attributes associated with it (Singh and Ellis, 1993). Singh and Ellis (1993) cited five studies that specifically compared Brussels sprout genotypes for resistance to cabbage aphid and found that six Brussels sprout cultivars and two clones demonstrated cabbage aphid resistance. Way and Murdie (1965) found antixenosis of Brussels sprouts to be associated with a glossy genotype and with low wax content. Ellis et al. (2000) demonstrated that four wild *Brassica* species showed high levels of antibiosis to cabbage aphid. Future *Brassica* breeding programs could possibly use these beneficial genetic resistance characteristics to incorporate in an integrated pest management scheme.

Destruction of host crop residue. Cabbage aphid eggs overwinter on *Brassica* plant residue that has not been plowed into the soil. One of the cultural practices that may reduce cabbage aphid populations is the destruction of crop host residues; however, to the best of our

knowledge, studies evaluating the efficacy of destroying crop residue have not been published in the scientific literature Wild *Brassica* relatives may persist in the surrounding environment and Shah et al. (2004) report that cabbage aphid can overwinter and live on alternative hosts beyond *Brassica* species.

Color of insecticide. The color of insecticide materials that cover the host plant may also have an effect on the control of cabbage aphid (Moore, 1937; Painter, 1951). Cabbage plants treated with an insecticide mixed with charcoal for black coloring had less than half the number of cabbage aphids compared to plants treated with a white insecticide (Painter, 1951). Moore (1937) also found that cabbage sprayed with insecticides dyed different colors showed significant differences in number of cabbage aphid. Plants with uncolored, white insecticide had fewer cabbage aphid than their red, green or black counterparts (but black had the least aphids of the last three). There is potential for the addition of inert or compatible ingredients that alter the color of already moderately effective insecticides to increase efficacy.

Light intensity. Painter (1951) concluded that differences in light intensity reflected from the leaves in the colored insecticide treatments was responsible for the differences in aphid numbers. Gabrys (2008) state that aphids in flight respond to shape, size, and density of potential host plants. Cabbage aphid are particularly attracted to the wavelength 550-590nm (Gabrys, 2008). Host plants are more easily located by winged aphids if they are grown in bare soil which allows for light reflection contrast between the plant and the soil background (Gabrys, 2008). Colored and reflective mulches are commercially available for field production of vegetables and could also be a potential option for aphid control, but material cost and disposal of these products should be considered. Furthermore, natural enemies of aphids may also be affected.

Plant spacing. Way et al. (1996) found that spacing of Brussels sprout plants affected the number of cabbage aphid on plants treated with the same amount of soil-applied pesticide (menazon) per plant. Plants with 36-inch spacing within row had fewer cabbage aphid than plants with 18-inch spacing.

Overall aims, objectives and hypotheses

There are many insecticides labelled for use in managing cabbage aphid, however, there are limited data about the efficacy of organic-approved, commercially available insecticide materials against cabbage aphid. Use of alyssum insectary intercropping has been successful for control of aphids in California (Brennan, 2013) but use of this practice is untested locally where populations of natural enemies may be different. These gaps in the literature, compounded with local crop losses (Levy and Sideman, unpublished) beg for more integrated pest management strategies against this persistent pest.

Our overall goal was to explore the relationship between Brussels sprout and cabbage aphid in our local agroecosystem and find an integrated approach to managing this pest using chemical and biological pest management strategies in conjunction. We had three specific objectives. Our first objective was to evaluate four organic insecticide treatments for their ability to control cabbage aphid. Our second objective was to evaluate seven species of insectary plants in the field for their ability to attract predators and parasitoids of cabbage aphids. Our third objective was to determine whether predation and parasitism of the cabbage aphid varied with proximity to insectary plants.

We hypothesized that one or more of the organic-approved insecticide treatments tested would provide a statistically significant decrease in cabbage aphid as compared with the untreated control, as measured either by number of aphids on leaves or percentage of infested

sprouts. Our second hypothesis was that different species of insectary plants would result in different hoverfly densities and that number of other insects observed would selectively visit certain insectary plant species. Our third hypothesis was that increased proximity to insectary plants would increase predation and parasitism of cabbage aphid.

CHAPTER 2:

CONTROL OF CABBAGE APHID USING INSECTICIDES

INTRODUCTION

As described in chapter 1, there have been surveys conducted in 2017 (Levy and Sideman, unpublished) and 2019 (Scheufele, unpublished) in which commercial farmers reported crop losses from cabbage aphid on *Brassica* crops. Most of the farmers have organic certification or use reduced-risk methods in their operations (Scheufele, unpublished), which suggests that certified organic and low-input growers are having trouble managing this pest with the tools available to them. Insecticides are a common form of control for cabbage aphids around the world (Bahana and Karuhize, 1986; Ellis et al., 2000) and locally in the Northeast United States. This chapter focuses on insecticides that are permitted for use in certified organic systems and will be referred to as "organic-approved" insecticides. Currently, efficacy reports of "organic-approved" insecticides that control cabbage aphid are sparse, and farmers are discouraged from growing Brussels sprouts (Levy and Sideman, unpublished).

Most organic-approved insecticides must directly smother or come in contact with the mostly stationary cabbage aphid; thus, good coverage is essential to ensure that insecticides, regardless of mode of action, reach the pest. Local crop reference guides list a plethora of organic pesticides for treatment of aphid (McKeag and Dicklow, 2017; Seaman, 2016), but this chapter will focus on products with the following active ingredients: azadirachtin, pyrethrins, potassium salts of fatty acids, and entomopathogenic-fungi spores.

Neem products (Azadirachtin). Products from the neem tree (*Azadirachta indica* (Meliaceae)) are used as botanical insecticides. Girish and Shankara (2008) report that insecticidal properties of neem are the most effective among 2,400 plant species tested for such properties. Azadirachtin is the primary and most recognized biologically active constituent of neem responsible for decreased numbers of insects (Henn and Weinzierl, 1989; Walter, 1999). However, other minor liminoid constituents of neem such as meliantriol, salannin, nimbandiol, nimbin, and deacetyl nimbinbandiol (Walter, 1999) are also biologically active and influence the activity of azadirachtin (Ahmed and Grainge, 1986; Henn and Weinzierl, 1989; Walter, 1999). One neem-based product, Neemix, contains nimbandiol, deacetylsalannin, deacetylnimbin, nimbin, 6-acetylnimbandisol, and salannin constituents, which have demonstrated synergistic activity (Walter, 1999). These constituents are not efficacious against insects on their own but have been demonstrated to improve the efficacy of azadirachtin (Walter, 1999). Moreover, the evolution of pest resistance against a complex of active ingredients in an insecticide may also be slower than that of a single pure compound insecticide (Pavela, 2006). Furthermore, "original" essential oil complexes have been found to be more effective in the control of pests than single pure compounds (Walter, 1999). Muhammad et al. (2018) used an original essential oil complex and reports that neem seed extract, turmeric (*Curcuma longa*) rhizome extract, and synthetic pyrethroid-based pesticides (Cypermethrin and Bifenethrin) reduced aphid populations in okra (*Abelmoschus esculentus*). Plants treated with neem seed extract were found to have fewer aphids than plants treated with the synthetic pyrethroid insecticides, which were not statistically different than plants treated with turmeric rhizome extract.

Despite the attempt to use concentration of azadirachtin as a marker for insecticidal activity of these mixtures, there is no standardization of neem-based products (Walter, 1999).

There are differences in the extraction process, formulation of solvents, and other adjuvants or "inert" ingredients that affect insecticidal properties of neem-based insecticides (Walter, 1999). These differences make comparing insecticide efficacy difficult (Walter, 1999) and since neem is a mixture of multiple active compounds, it is hard to clearly identify the precise mode of action in various neem products that are prepared differently.

Current literature shows that neem is a secondary feeding deterrent and growth regulator that causes insects to stop feeding and can interrupt reproductive maturity (Ahmed and Grainge, 1986; Henn and Weinzierl, 1989; Mordue (Luntz) & Nisbet, 2000; Mordue (Luntz) et al., 1998). Primary anti-feedant properties of insecticides deter insects from ingesting the crop from the beginning, whereas the secondary anti-feedant properties of azadirachtin result post-ingestion. Aphids that have ingested azadirachtin experience a reduction of food consumption and digestive efficiency (Mordue(Luntz) & Nisbet, 2000). A laboratory study reported that aphids slowed their feeding rate following 24 hours of a diet comprised of 25ppm azadirachtin (Nisbet et al., 1994). Azadirachtin terminates insects slowly in part by disrupting their molting cycle necessary for development, which in turn causes them to perish. Thus, it has been shown that azadirachtin does not cause mortality to mature aphids (Pavela et al., 2004). Since the kill time is slow, low-functioning insects may remain on the crops, however, since azadirachtin has secondary anti-feedant properties pests may feed less on the crop. In this way, counting number of insects prior and after foliar application may still show a similar number of pests, but the damage to the crop may not continue (Walter, 1999). Some neem products may be used in rotation with adulticides or practices that encourage conservation of beneficial insects that can parasitize or predate on adult pests (Walter, 1999).

Researchers have studied the effects of different concentrations of neem oil extract versus azadirachtin on aphids. Opender (1998) tested various levels of neem seed oil extract (1%, 1.5%, 2%) compared with different concentrations of azadirachtin (30ppm and 60ppm). They found 49% to 70% fewer cabbage aphid offspring were produced in all insecticide treatments than in the water control treatment. Reduction in offspring was greatest in 2% neem seed oil and 60ppm azadirachtin. Since Opender (1998) found similar results with both neem seed oil and pure azadirachtin, they attribute the reduction of cabbage aphid fecundity found in their experiment specifically due to the azadirachtin component of neem. Mordue (Luntz) et al. (1998) and Nisbet et al. (1994) confirmed that specifically the azadirachtin constituent of neem is what interferes with the reproduction of aphids. Female aphids fed a diet with 5ppm of azadirachtin had a significant decrease in fecundity within 48 hours (Mordue (Luntz) et al., 1998). Furthermore, aphids fed a diet with 10ppm azadirachtin produced young that were not capable of surviving (Mordue Luntz et al., 1996). Adult green peach aphids (*Myzus persicae*) that were fed 25-100ppm azadirachtin for 26 hours slowed their asexual reproduction rate. After 50 hours of the diet nymph production had essentially halted or the nymphs produced did not reach sexual maturation (Nisbet et al., 1994).

Studies evaluating azadirachtin had some effectiveness in controlling aphids, however, efficacy is dependent on aphid species and formulation of the insecticide mixture. Effects of azadirachtin concentration levels vary according to aphid species. To inhibit reproduction, the black bean aphid (*Aphis fabae*) require 3-3.5ppm azadirachtin (Dimetry and Schmidt, 1992) and the cabbage aphid require 60ppm azadirachtin (Opender, 1998). The lettuce aphid (*Nasonovia ribisnigri*), the strawberry aphid (*Chaetosiphon fragaefoli*), and the green peach aphid require 60-80ppm azadirachtin to inhibit reproduction (Lowery and Isman, 1996). Nisbet et al. (1992)

reported a requirement of over 100ppm azadirachtin to induce antifeedant effects on green peach aphid. Currently, field studies on azadirachtin products specifically against cabbage aphid are lacking in the literature. Three out of four experiments using azadirachtin-based products had efficacy in the control of other aphids, whereas four out of seven experiments controlled green peach aphid (Seaman, 2016).

Use of adjuvants or synergists may increase the efficacy of neem-based products against aphids in the field. Seaman (2016) suggests mixing azadirachtin products with an oil. Walter (1999) cite a field study demonstrating synergistic activity for increased efficacy against aphids (rosy apple aphids (*Dysaphis plantaginea*) and green peach aphids) when Neemix (neem) is mixed with stylet oil or M-pede (Potassium salts of fatty acids). The increased efficacy of Neemix mixed with stylet oil or M-pede was attributed to the insecticide mixture drying slower than Neemix alone (Walter, 1999). Mohan et al. (2007) showed that neem can have synergistic effects with specific strains of biological insecticides. Neem also carries antifungal properties (Girish and Shankara, 2008; Henn and Weinzierl, 1989) which could be advantageous against phytophagous fungi, or could potentially be detrimental towards beneficial fungi.

The effects of neem seed oil on natural insect enemies of aphids have been studied in the laboratory. Lowery and Isman (1995) tested different concentrations of neem seed oil (0.5%, 1.0% and 2.0%) for their effect on hoverfly (*Eupeodes fumipennis* (Thompson)), ladybeetle (*Coccinella undecimpunctata* L.), and parasitic wasp (*Diaeretiella rapae* (Mclntosh)). *Coccinella undecimpunctata* L. were severely affected; there was 100% mortality of larvae in all concentrations of neem seed oil; they were unable to pupate or eclose (emerge from pupa). *Eupeodes fumipennis* were not as sensitive, but still had a reduction in eclosion on neem oil seed treatments. *Diaeretiella rapae* (Mclntosh) rate of aphid parasitism was not reduced on neem

seed oil treated plants indoors as well as in the field (Lowery and Isman, 1995). Despite some detrimental effects of neem seed oil on some beneficial insects in the laboratory, Lowery and Isman (1995) conclude that use of neem products in field conditions are relatively benign to their natural enemies. Schauer (1985) found parasitoid wasps that had been sprayed with azadirachtin still hatched at the same rate of their unsprayed counterparts. If beneficial populations are diminished by broad spectrum insecticides, secondary pest outbreaks can occur (Walter, 1999). Walter (1999) concludes that the low impact of azadirachtin on natural enemies allows for it to be used as an integrated pest management tool in conjunction with biological control.

Systemic properties of insecticides may reduce exposure to natural insect enemies of aphids. Neem can be taken up by the roots of plants (vegetables and trees) and translocated to other parts of the plant as natural metabolites and act as a systemic insecticide (Basedow et al., 2002; Henn and Weinzierl, 1989; Pavela et al., 2004; Sundaram, 1996). Nisbet et al. (1992 and 1993) concur that when azadirachtin is used systemically it has antifeedant effects on green peach aphid. Pavela et al. (2004) found longevity of nymphal stage was inversely related with azadirachtin concentration at plant roots; as azadirachtin concentrations increased, longevity of nymphal cabbage aphid decreased. However, mature cabbage aphid longevity was not affected, regardless of concentration (Pavela et al., 2004), which was confirmed by Nisbet et al. (1994). Pavela et al, 2004 hypothesizes low concentrations of azadirachtin or botanical insecticides applied systemically could be more efficacious for control of pests compared to foliar application. When applied to the foliage, azadirachtin effectively terminated Birch leafminer (*Fenusa pusilla*) through the leaf cuticle (Larew et al., 1987), but did not demonstrate translocation from leaf to leaf (Walter, 1999).

Pyrethrum, pyrethrins, pyrethroids. Pyrethrum is the dried flowerhead of *Chrysanthemum cinerariaefolium* that has been ground into a powder (Henn and Weinzierl, 1989). Pyrethrins are six insecticidal compounds that are extracted from the ground flower powder and used to manufacture insecticide materials (Henn and Weinzierl, 1989). Pyrethrins are more concentrated, since they constitute only 0.9-1.3% of the flowerheads themselves. Pyrethroid insecticides are synthetic compounds; they are not botanical insecticides (Henn and Weinzierl, 1989) and are not approved for organic production. In contrast to pyrethrins, pyrethroids are more persistent in the environment, more toxic to insects, and effective at very low concentrations (Henn and Weinzierl, 1989).

Pyrethrins **mode of action** is through the cuticle of the insect. Pyrethrins disrupt ion exchange in nerve fibers and interrupt the regular transmission of nerve impulses. In turn, the nervous system of insects become rapidly paralyzed by toxicity and they sometimes die (Henn and Weinzierl, 1989). Despite initial acute toxicity, many insects can metabolize and detoxify pyrethrins and may recover, rather than die. Piperonyl butoxide (PBO) is a common synergist ingredient in pyrethrin products that increases their efficacy by preventing insect recovery and survival (Henn and Weinzierl, 1989). However, since PBO is a synthetic compound it is not approved for use in certified organic agriculture.

Studies evaluating pyrethrin products demonstrated some efficacy in controlling aphids, however, different aphid species affected product efficacy. In a review article, Seaman (2016) cites the organic-approved insecticide PyGanic EC 1.4 II (pyrethrins) to be effective in older experiments against cabbage aphid. However, only one out of the three experiments using pyrethrin products cited had efficacy in the control of green peach aphid. Pyrethrum was effective in the control of aphid on artichoke, however, was ineffective against aphids on spinach

and commercial greens (Casida, 1980). Up to date field studies on pyrethrins against cabbage aphid on Brussels sprouts are lacking in the literature. Since repeated used of synthetic pyrethroids can lead to insect resistance to pyrethrins (Casida, 1980), efficacy of these products against insects may change over time.

Lab experiments reported that pyrethrin products evaluated on beneficial parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez), and beneficial lady beetle *Adalia bipunctata* (L.) had 100% mortality (Jansen et al., 2010). Their results showed that pyrethrin products are potentially very toxic to natural enemies of aphids in a lab setting, however, literature does not confirm this finding in the field setting.

Potassium salts of fatty acids (M-pede). Other names that may refer to potassium salts of fatty acids are "soap salts" (Dheeraj et al., 2013) or "insecticidal soaps." Potassium salts of fatty acids are made by mixing potassium hydroxide with fatty acids (from animal fats or plant oils) (Dheeraj et al., 2013; Sy Mohamad et al., 2013). Potassium salts of fatty acids are used as herbicides, fungicides, and algaecides (Dheeraj et al., 2013). Oleic acid, a fatty acid found in high concentrations in olive oil has been shown to have high insecticidal properties. Safer® soaps are trade name of potassium salts of fatty acids that are commercially available and according to Henn and Weinzierl (1989) the active ingredient is potassium salt of oleic acid. However, the current label of both Safer® Soaps and M-Pede reads "Potassium salts of fatty acids", thus, comparisons of different fatty acids cannot be readily made.

The **mode of action** is dependent on direct contact with the pest, which is a physical control that smothers the insect, rather than chemical insecticidal properties. When potassium salts of fatty acids contact the surface of the pest, the cuticle (outer coating of the aphid) is penetrated (Henn and Weinzierl, 1989), spiracles (responsible for air exchange) are obstructed

and the insect is fatally suffocated (Dheeraj et al., 2013; Sy Mohamad et al., 2013). Potassium salts of fatty acids work on most soft-bodied insect pests that do not have thickened cuticles (Henn and Weinzierl, 1989) and have been successful in the control of aphids (Dheeraj et al., 2013; Sy Mohamad et al., 2013; Wafula et al., 2017). For the soap to be effective, however, the material must contact the pest body while it is still in liquid form; once the material has dried, it no longer has insecticidal effects and degrades quickly (Henn and Weinzierl, 1989). Jansen et al. (2010) concluded that potassium salts of fatty acids were not harmful to mobile natural enemies of aphids (parasitic wasps and lady beetles) and indicated it as a selective, safe alternative to other insecticides labeled for aphids that rely on chemical insecticidal properties.

Studies evaluating soap products had some effectiveness in controlling aphids, however, aphid species appears to be important. Six out of eight experiments using soap products had efficacy in the control of "other" aphids, whereas zero out of nine experiments controlled green peach aphid (Seaman, 2016). Currently, field studies on soap products against cabbage aphid and are lacking in the literature. Seaman (2016) suggests using M-pede (potassium salts of fatty acids) in combination with another labeled product, however, literature that shows increased efficacy with these mixtures or explanation of possible synergism is lacking.

Entomopathogenic fungi-based insecticides. Entomopathogenic fungi are parasitic to insects. If conditions (i.e. relative humidity, temperature) are correct the spores will germinate and consume the body of the insect (Reyes-Rosas et al., 2012). There are commercialized "biological insecticides" that have entomopathogenic fungi spores as their active ingredient. The specialized mouth part of the aphid does not allow for the ingestion of insect-harming bacteria or viruses to enter the aphid body as a biological control (Shah et al., 2004) but fungal spores can penetrate through the cuticle (outer coating) of the aphid (Reyes-Rosas et al., 2012).

A strain of *Lecanicillium muscarium*, previously known as *Verticillium lecanii*, is commercially available under the tradename Mycotal. However, this specific biological insecticide material is not currently available in the United States. Mycotrol ESO (active ingredient *Beauveria bassiana*) is a commercially available entomopathogenic fungi-based insecticide labeled for the control of aphids but has not been tested for efficacy of cabbage aphid in the Northeast. Environmental factors play a large role in the success of entomopathogenic fungi (Reyes-Rosas et al., 2012) which makes it challenging to evaluate entomopathogenic fungi-based insecticides in field settings with changing environmental conditions.

There are many materials that are listed for the control of aphids, however, there are limited experiments that investigate organic-approved, commercially available insecticide materials against cabbage aphid. These gaps in the literature, compounded with local crop losses (Sideman, personal communication) beg for more organic-approved insecticide efficacy studies on cabbage aphid. Therefore, the objective of this chapter was to evaluate four organic-approved insecticide materials (Azera, AzaGuard, M-pede, Mycotrol ESO) over three years to compare their efficacy against cabbage aphid in Brussels sprout in field conditions in Durham, NH.

MATERIALS AND METHODS

Seedling production. Prior research has identified ideal planting dates, cultivars, and topping dates for Brussels sprouts (Sideman and Saunders, 2015) that were used in the design of this experiment. 'Diablo' Brussels sprout seeds were purchased from Johnny's Selected Seeds (Winslow, Maine). Brussels sprouts seeds were sown on 20 May 2016, 24 May 2017, and 31 May 2018 into 128 plastic cell trays using Promix BX (Pro-Mix, Quakertown, PA) soil-less media. Seeds were sown one seed per cell in 2016 and 2018. Seeds were sown two seeds per cell tray in 2017 and seedlings were thinned on 2 Jun 2017 at cotyledon stage with the first true leaf emerging. Seedling trays were fertilized with water soluble fertilizer two times each year before transplant [15N–2.2P–12.5K] (Peters Professional 15–5–15 Cal–Mag; Everris Intl., Geldermalsen, The Netherlands) at a rate of 300 ppm N.

Field site preparation and transplanting. Experiments were conducted in the same field in 2016, 2017 and 2018 at the University of New Hampshire at Woodman Horticultural Farm in Durham, New Hampshire, United States (43.150591ºN latitude and 70.942150º long). Prior to transplanting into the field, 150lbs/acre of nitrogen (N) as 27-0-0 and 50lbs/acre of K_20; (potash) as KCl (Potassium chloride (0-0-60)) were incorporated in the spring on all three years, based on soil test recommendations. Raised beds were created with 1 line of drip tape buried 1 inch below the soil surface. In 2016, raised beds were covered with 1 mil embossed plastic mulch, and for the following two years, with 0.6 mil Organix A.G. Film biodegradable black plastic mulch (Organix Solutions, Phoenix, Arizona). Brussels sprouts were transplanted on 21 Jun 2016, 16 Jun 2017, and 21 Jun 2018 into the field at the 5-leaf stage with healthy root development but before becoming root-bound. Brussels sprouts seedlings were planted at 18-inch spacing with six feet between rows. Plants were replaced when lost due to pest damage until three weeks after

transplant in all years. Applications of Dipel® DF were made throughout the growing season to combat cutworms, imported cabbageworm, diamondback moth, cabbage looper and salt marsh caterpillar pests. There was not much success with controlling the cutworms, which were dug out by hand and the Brussels sprouts seedlings were replaced when the stem was girdled. Brussels sprouts were topped (the apical meristem was removed) on the week of September 15 and harvested in November after a few hard frosts in all years.

Irrigation. The timing of irrigation events was determined by regular evaluation of the root zone. A clump of soil and the squeeze test was used to determine soil moisture (Healy, 2012). If a ball or clump of soil could be formed, no water was added. If the soil was loose and falling apart, the drip irrigation was turned on for an hour interval and the root zone was re-evaluated for moisture. Throughout the course of the season bio-degradable mulch did start to rip and fray but did not seem to negatively affect the plants. The bare soil exposed by rips needed more irrigation during sunny weather, but during rainy and moist periods allowed the overhead water to penetrate the soil bed. On average, the drip irrigation ran about four hours weekly in 2016, 1.5 hours weekly in 2017, and only run twice for two hours over the course of the entire season in 2018 due to regular rainfall.

Insecticide treatments. Choice of insecticide treatments was decided after gathering information from crop references (Seaman, 2016), discussions with growers (Levy and Sideman, unpublished), researchers and entomologists in the region regarding insecticide efficacy.

In 2016 our insecticide treatments for cabbage aphid were Azera ([azadirachtin and pyrethrins] MGK, Minneapolis, MN) and M-pede used in rotation ([Potassium salts of fatty acids] Gowan Co, Yuma, AZ) against a control. For this experiment, the control plot did not have any cabbage aphid insecticides applied to it. No sprays were applied was applied to these

plots, except for an insecticide to manage lepidopteran pests, which was applied to the entire field on all treatments in all three years. All plots were periodically sprayed with Dipel® DF ([*B. thuringiensis* var. kurstaki] Valent BioSciences, Libertyville, IL) per label recommendations for control of lepidopteran caterpillar pests that are not the focus of this experiment. Applications rates ranged from 0.5lbs/acre when plants were young to 2lbs/acre when plants were larger, as greater amounts of mixed material was needed to ensure good coverage.

In 2017 our three pesticide treatments for cabbage aphid were: (1) **AzaGuard** ([azadirachtin] BioSafe Systems LLC, East Hartford, CT), (2) **Azera** ([azadirachtin and pyrethrins] MGK, Minneapolis, MN) and (3) **M-Pede** ([Potassium salts of fatty acids] Gowan Co, Yuma, AZ) against (4) an untreated control (Table 1). The control was not water treatment; nothing was applied to these plots except for control of lepidopteran pests, which was applied to the entire field. Nu Film-P ([Poly-1-p-Menthene] MKG, Minneapolis, MN), a "spreader-sticker" adjuvant, was included in the Dipel® DF mixture as well as the AzaGuard treatment per label recommendations. The Dipel® DF+Nu Film P mixture was applied using a high velocity cannon sprayer Jacto J400 (Jacto, Tualatin, OR, USA).

In 2018, the same treatments as 2017 were used, and another insecticide treatment was added: (5) **Mycotrol ESO** (LAM International Corporation; Butte, MT [*Beauveria bassiana* strain GHA]) (Table 1).

Table 1. Insecticide treatments used against cabbage aphid: tradenames, manufacturer, location, active ingredient, and rate of insecticide concentrate used per 3 gallons of water. Azera and M-pede were used in rotation in 2016. Azera, M-pede, AzaGuard + Nu Film P were used without rotation in 2017. Azera, M-pede, AzaGuard + Nu Film P, and Mycotrol ESO were used without rotation in 2018. Rate of concentrates are from label recommendations. Since rate of concentrates are recommended as a range, the price per application reflects the same range. Prices are sourced from an average of available online prices without including shipping.

Insecticide Treatments

Insecticide Material	Manufacturer and Location	Active Ingredient	Rate of concentrate		Price range per application per acre
			per 3 gallons water	per acre	
Azera	MGK, Minneapolis, MN	azadirachtin and pyrethrins	177 mL	539mL-946mL	$65.5-$115
M-Pede	Gowan Co, Yuma, AZ	potassium salts of fatty acids	207 mL	1892mL	$23
Mycotrol ESO	LAM International Corporation; Butte, MT	*Beauveria bassiana* Strain GHA	88.7 mL	237mL-946mL	$25 - $99
AzaGuard	BioSafe Systems LLC, East Hartford, CT	azadirachtin	28.5 mL	295mL-473mL	$50 - $79
Nu Film P (adjuvant mixed with AzaGuard)	MKG, Minneapolis, MN	Poly-1-p-Menthene	4.9 mL	61mL - 121mL	$1 - $2

Application timing. Decisions regarding insecticide application timing were made based on the sequential sampling protocol for economic thresholds for cabbage aphid published by the University of California Cooperative Extension (Natwick, 2009). Based on number of data plants per plot, we sprayed when thresholds reached 14% of plants with at least one cabbage aphid per plot, compared to the recommended 15%. Pesticide application decisions based on economic thresholds were also in alignment with specimen label recommendations at eight to 10-day intervals . Each plot was considered individually to determine economic damage thresholds per plot and insecticide treatments were only applied if the specific plot reached economic threshold, regardless of the other replications of the same treatment. In 2016, treatments were rotated between M-pede and Azera. In 2017, all spray treatment rates and mixtures remained the same throughout the growing season; sprays were not rotated. Bamboo stakes (5ft) with colored flags were installed to delineate between cabbage aphid insecticide treatment plots and the untreated plants.

Experimental design. A randomized complete block design was used in all three years. In 2016 there were four replications of two treatments with a total of eight insecticide and untreated plots. Each plot was comprised of 39 plants, in three rows of 13 plants. In 2017 there were three replications of four treatments with a total of 12 insecticide and untreated plots. Each plot was comprised of 36 Brussels sprouts plants, in three rows of 12 plants. In 2018, there were three replications of five treatments with a total of 15 insecticide and untreated plots. Each plot was comprised of 30 Brussels sprout plants, in three rows of 10 plants. In all years, treatment plots were surrounded with border of untreated Brussels sprout plants.

Data. In 2016, six Brussels sprout plants were sampled; two plants from each row were chosen at random for aphid counts. For both 2017 and 2018, nine plants per plot were sampled; three plants from each row were chosen at random for counting insects on their leaves. In all three years, six leaves per plant were observed to count insects on both sides of the leaves. For each plant, two leaves each from the lower, middle, and upper portion of the plant were observed for aphid count. In 2016 there were seven observations, averaging every 14 days from 3 Aug 2016 to 14 Nov 2016. In 2017 there were 11 observations, averaging every 10 days from 19 Jul 2017 to 2 Nov 2017. In 2018 there were 11 observations averaging every 9 days from 29 Jul 2018 to 8 Nov 2018.

At harvest, we selected six plants (in 2016) or eight plants (in 2017) from the middle row of each plot (Figure 3). All leaves were removed to view the "sprouts" (the axial buds; the portion that is eaten). Sprouts were observed superficially while still on the stalk and percentage of buds affected by cabbage aphid was determined per stalk. In 2016, a sprout was considered "infested" if there were enough aphids that peeling off the outer leaves of the sprout bud would not clean it entirely. A sprout was considered to have "few" aphids if there were superficial aphids that could easily be cleaned off the outside of the sprout. A "clean" sprout had no aphids from the outside view. In 2017 there were no aphids on the outside of buds, which would have resulted in 100% "clean" sprouts under the 2016 definition but instead a closer method of inspection was used to discern differences between treatments. Moreover, 10 of the Brussels sprout buds were sampled throughout the stalk (two in each quarter section of the stalk length) by pulling back the outer leaves of the bud to inspect for dead or alive aphids. If there were one to five aphids (dead or alive) on the inside of the outer-most leaves of the sprout, there were

considered to be “few aphids” (Figure 4). Sprouts that were considered “clean” had to have zero dead or live aphids found when outer leaves of sprouts were pulled back.

Figure 3. Final harvest of the eight Brussels sprouts stalks in the center of each insecticide treatment plot in 2017.

Figure 4. A sprout with “few” aphids in 2017. The outer leaves of the Brussels sprout buds were pulled back to look for dead or live aphids. One to five dead aphids were considered “few aphids.”

Data analysis. Printed Excel spreadsheets and field note books were used to collect data when scouting for insect populations. Notebook data were entered into an Excel spreadsheet. We calculated the average percent infested sprouts per stalk for each replicate and summed aphid presence on leaves to evaluated difference in insecticide treatments. Then, using JMP Pro 13 statistical software, we tested for and confirmed normal distribution which was already present in both leaf count and percentage data. Therefore, no transformation was used. An Analysis of Variance (ANOVA) was conducted. Tukey's honestly significant difference (HSD) was used to calculate means separation at α=0.05 for leaf count data.

RESULTS

Count of cabbage aphid on Brussels sprouts leaves over time.

Year one: 2016. In mid-July the first winged aphids began to fly into the field, distributed in patches throughout the field (Figure 5). Plots where Azera and M-pede were applied in rotation had significantly fewer cabbage aphids than the control plots from 21 Sept 2016 to 4 Nov 2016 (Figure 5). Aphid numbers in the control plot continued to increase until 4 Nov 2017, the last sample date before harvest (Figure 5).

Year two: 2017. In mid-July the first winged aphids were observed in the field, distributed in patches throughout (Figure 6). The first winged cabbage aphid was found on down-wind edge of the field. Cabbage aphid numbers increased in all treatment plots until mid-September. At peak aphid populations on 28 Sept 2017, plots treated with AzaGuard+ NuFilm P and Azera had significantly fewer cabbage aphids than M-pede and control plots (Figure 6). 28 Sept 2017 was the only sample date in 2017 where significant differences of aphid numbers were found between treatments. Following this peak, there was a substantial decrease in cabbage aphid numbers across all treatments (Figure 6).

Year three: 2018. Winged aphids did not reach the experiment plots until 27 July 2018. There were the greatest number of cabbage aphids on 8 Aug 2018 (mean of 0.22 aphids per six leaves) which resulted in only one third of the plots reaching economic threshold on that single date. Populations were not severe enough to compare insecticide treatments and final harvest in all plots resulted in 100% clean sprouts.

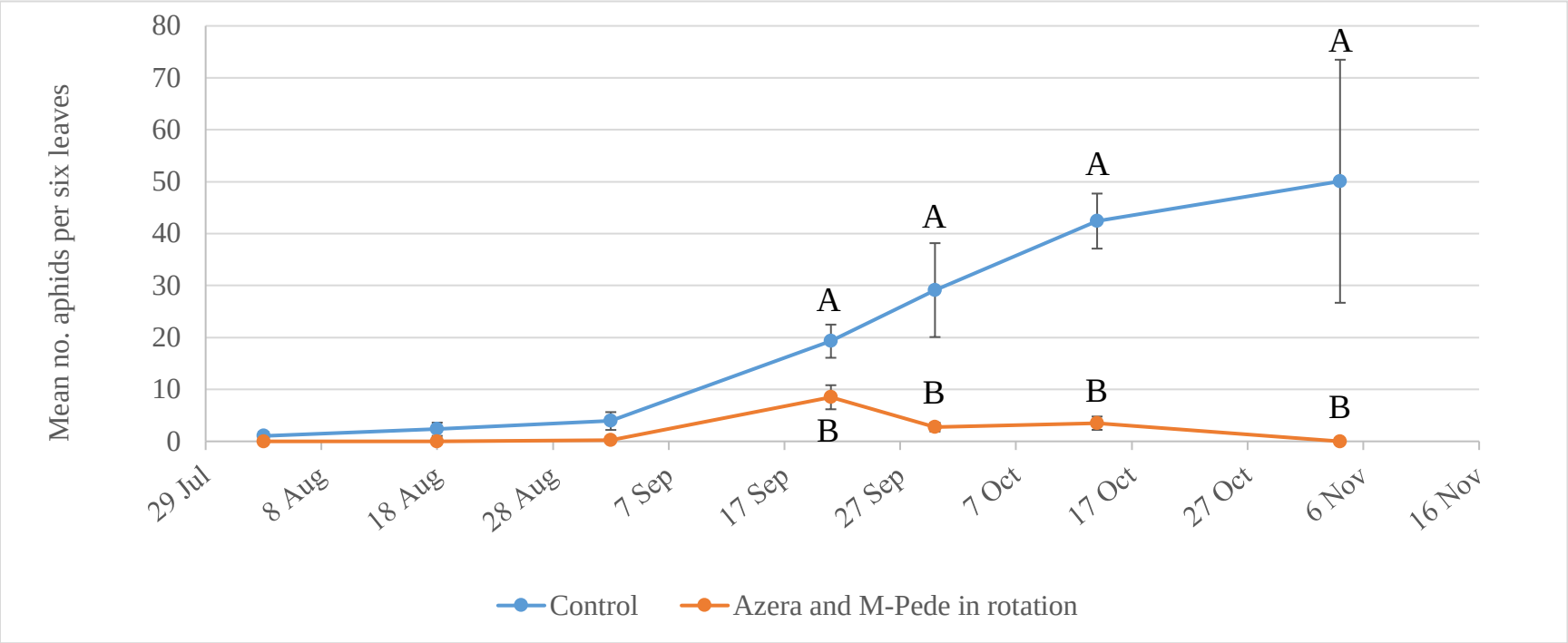


Figure 5. Number of cabbage aphids per six leaves in 2016. Azera and M-pede were applied in rotation; the control was unsprayed. Six Brussels sprouts plants were randomly selected per plot and cabbage aphids were counted on six leaves per plant. Cabbage aphid count was summed per six leaves and means are from 4 replicates. Error bars represent standard error. Each marker signifies one of the 7 sample dates from 3 Aug 2016 to 4 Nov 2016. Within date, treatment means followed by the same letter are not significantly different according to Student's T-test at α =0.05. On dates with no significant differences between treatment means there are no means separation letters.

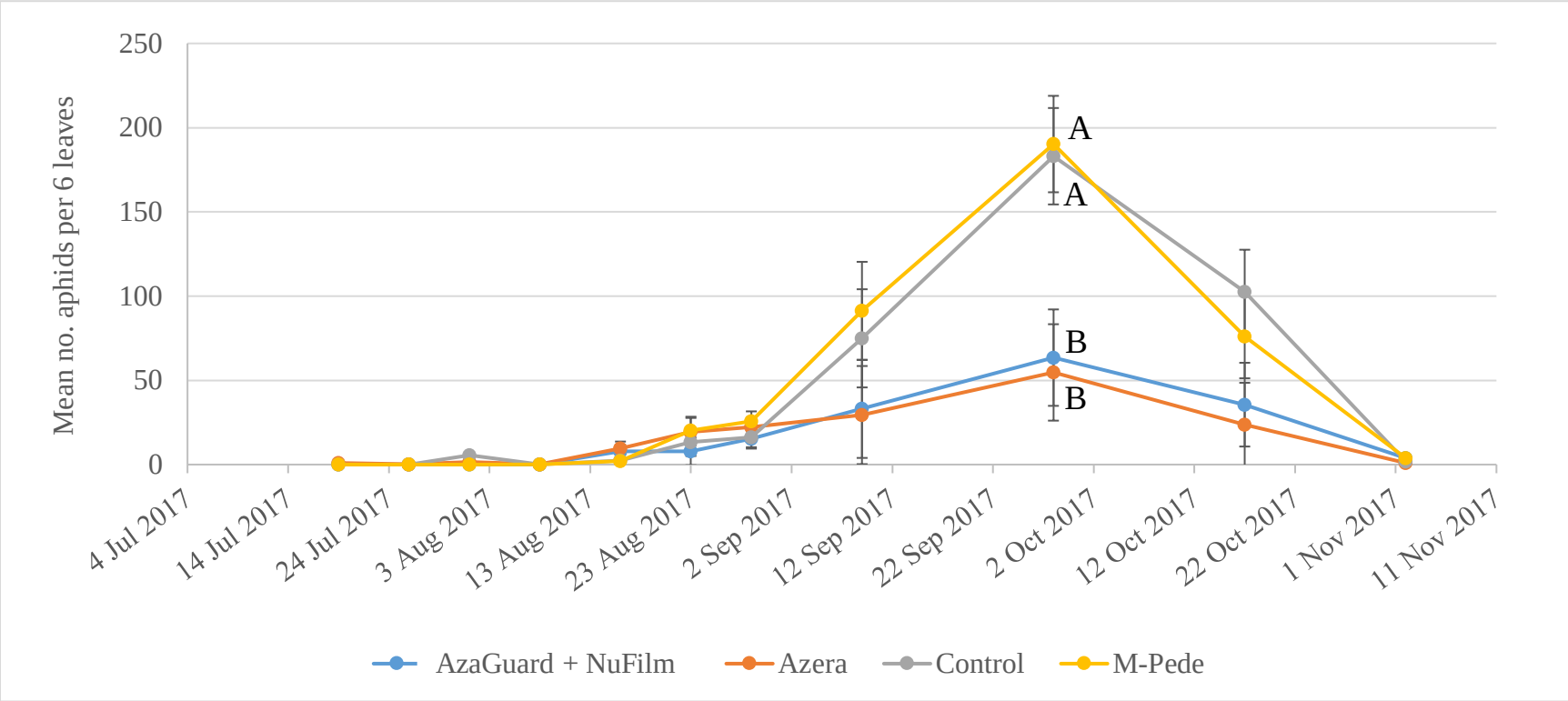


Figure 6. Number of cabbage aphids per six leaves in 2017. Control plots were unsprayed. Six Brussels sprouts plants were randomly selected per plot and cabbage aphids were counted on six leaves per plant. Cabbage aphid count was summed per six leaves. Means are from 3 replicates. Error bars represent standard error. Each marker signifies one of the 11 sample dates from 19 Jul 2017 to 2 Nov 2017. Within date, treatment means followed by the same letter are not significantly different according to Tukey's HSD at $\alpha = 0.05$. On dates with no significant differences between treatment means there are no means separation letters.

Economic thresholds

In **2016**, economic damage thresholds were first reached on 19 Jul 2016 and not again until about two months later. In **2017**, economic damage thresholds were reached on 14 Aug 2017 and were maintained in all plots until harvest. By mid-September every Brussels sprout plant within the experiment had a least one cabbage aphid. Aphids remained at damaging levels until aphid populations in all plots, including the control plot, plummeted after 23 Oct 2017.

Number of insecticide applications

In **2016**, cabbage aphid insecticide treatments were applied on eight dates, alternating between Azera and M-Pede (Table 2). In **2017**, cabbage aphid insecticides (Azera, AzaGuard+NuFilm P, M-Pede) were applied on seven dates as plots reached economic damage thresholds (Table 2). Dipel DF was applied six times in **2016** and Dipel DF + NuFilm-P was applied four times in **2017.**

Table 2. Application dates of insecticides to experimental plots in 2016 and 2017.

2016			2017	
Cabbage aphid insecticide applied [z]		**Dipel DF**	**Cabbage aphid insecticides**	**Dipel DF + Nu Film P** [y]
M-Pede	19 Jul	8 Jul	14 Aug	26 Jun
Azera	13 Sep	11 Jul	24 Aug	21 Jul
M-Pede	22 Sep	26 Jul	5 Sep	28 Aug
Azera	28 Sep	12 Aug	14 Sep	5 Oct
M-Pede	7 Oct	1 Sep	26 Sep	
Azera	12 Oct	9 Sep	12 Oct	
M-Pede	20 Oct		23 Oct	
Azera	2 Nov			
Number of applications per year	8	6	7	4

[z] Insecticides used to control cabbage aphid were rotated in 2016, but not in 2017.

[y]Dipel DF was used to control caterpillar pests. In 2016, no adjuvant was used with Dipel DF, whereas in 2017 Nu Film P was mixed as a wetting agent

Final harvest: effects of cabbage aphid on harvest

Final harvest 2016. The rotation of Azera and M-pede resulted in 10 times more sprouts that were not infested contrasted with the unsprayed control plots. The rotation of Azera and M-pede resulted in 91% of sprouts with no visible aphids on the outside ("clean"), 8% of sprouts with few superficial aphids that could easily be cleaned off ("few aphids"), and 1% of sprouts had enough aphids that peeling off the outer leaves of the sprout would not clean it entirely ("infested"). The unsprayed control plots resulted in only 9% "clean" sprouts, 58% "few aphids" on sprouts, and 33% "infested sprouts" (Figure 7). Data supported the hypothesis that insecticides would reduce aphid infestation compared the control.

Final harvest 2017. From the outside view of the Brussels sprouts stalk and sprouts there appeared to be adequate control of cabbage aphid in all treatments. There were zero percent "infested sprouts" in 2017. However, upon closer inspection by pulling back the outer leaves of the sprout, small numbers of mostly dead aphids were found. The definition of "clean" sprouts was then changed to zero presence of dead or live aphids on the inside or outside of a sprout. Though the mean percentage of clean sprouts treated with Azera (60%) and AzaGuard+NuFilm P (57%) were greater than those treated with M-Pede (39%) and the unsprayed control (41%), there were no statistical differences found between any of the treatments(p=0.099) (Figure 8). Data did not support the hypothesis that one or more insecticides would show statistically greater efficacy against cabbage aphid compared to the control.

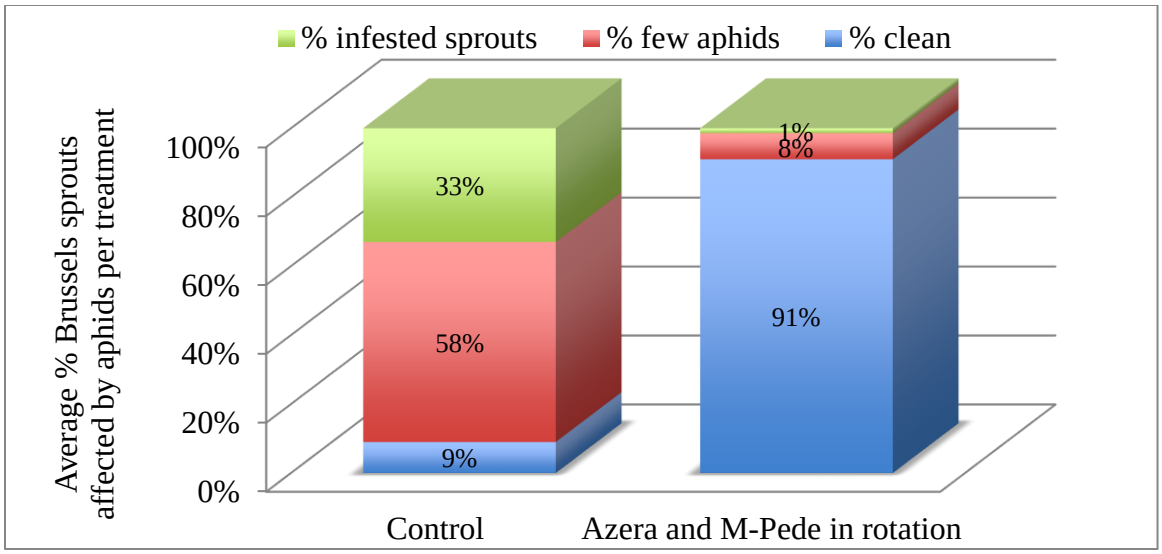


Figure 7. Final harvest: percent sprouts affected by cabbage aphid, 2016. Percent clean sprouts was significantly greater for Azera and M-pede in rotation than for unsprayed control treatment. "Infested sprouts" had enough aphids that peeling off the outer leaves of the sprout would not clean it entirely. "Few aphids" had superficial aphids that could be easily clean off outside of the bud. "Clean" sprouts had no visible aphids on the outside of sprouts.

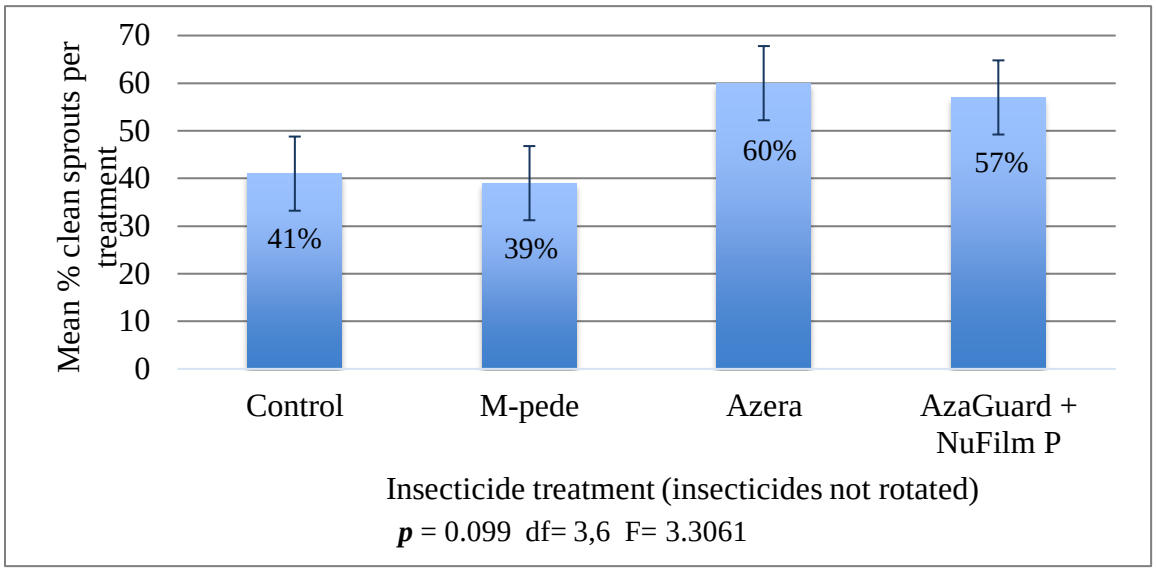


Figure 8. Final harvest: percent "clean sprouts" not affected by cabbage aphid, 2017. "Clean sprouts" had zero aphids on the inside or outside of sprouts. There were no significant differences between treatments. There were zero infested sprouts in 2017. The remaining percentages per treatment had "few aphids." "Few aphids" had one to five (live or dead) aphids on the inside of the outer leaves of the sprout.

Naturally occurring entomopathogenic fungus

In both 2017 and 2018, we observed naturally occurring entomopathogenic fungi on parasitized cabbage aphid colonies on Brussels sprouts in the field during the second week of October. On both years, we sent samples to specialists at the University of Vermont for identification. Using microscopic features, Agrin Davari and Margaret Skinner determined the entomopathogenic fungus found in 2017 was very likely to be *Neozygites fresenii* (Entomophthorales: Neozygitaceae) (Figure 9) and likely *Lecanicillium muscarium* (Hypocreales: Cordycipitareae) in 2018.

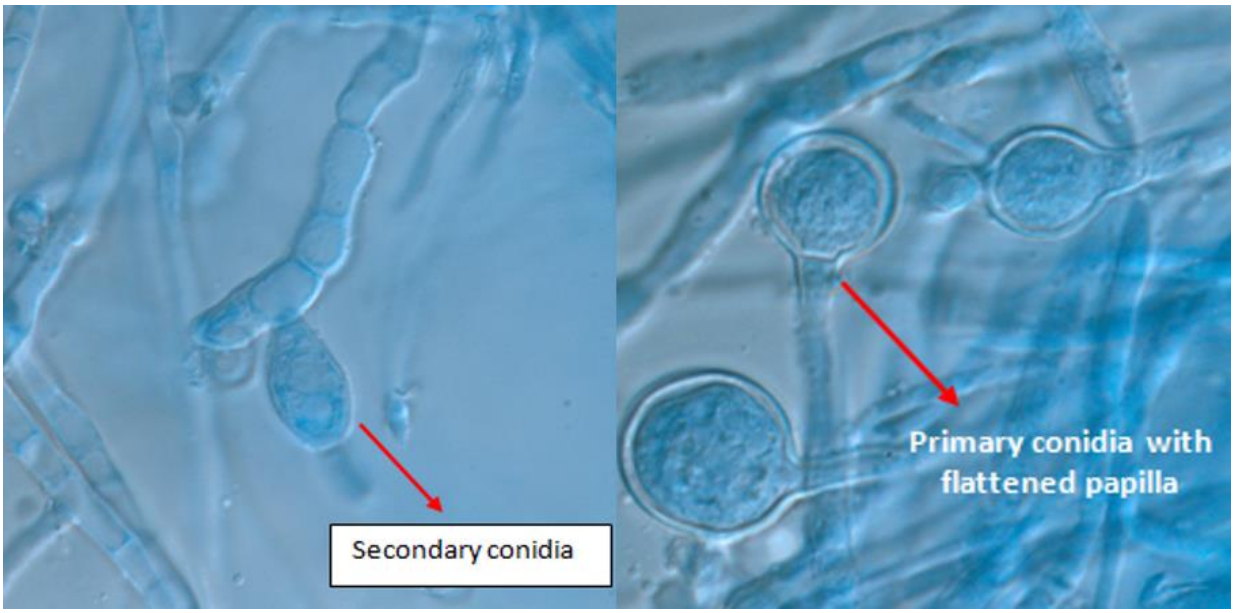


Figure 9. Microscope photographs of entomopathogenic fungus, likely Neozygites fresenii (Entomophthorales: Neozygitaceae) in 2017. Photographs by Agrin Davari and Margaret Skinner at the University of Vermont.

In 2017, there were very high numbers of cabbage aphid that collapsed suddenly in all treatments during the same period that entomopathogenic fungus was observed. Cabbage aphid numbers peaked on 28 Sept 2017, and it is likely that entomopathogenic fungi germinated in the humid environmental conditions of 7 Oct 2017 through 10 Oct 2017. It rained a small amount daily for an average of 0.38 inches, and the average number of hours with leaf wetness per day

was 10. The average number of hours with ≥ 90% relative humidity was 16.67, and windspeed averaged 2.87 miles per hour. The average air temperature per day over this period was 67.70°F. The next observation date of aphid numbers on Brussels sprout leaves was 17 Oct 2017 and live aphid numbers had decreased, while the dead bodies of numerous aphids were left attached to the leaf with fuzzy gray-black-brown fungal mycelium (Figure 10).

Figure 10. Entomopathogenic fungus as seen by the naked eye in 2017. Photograph by Alan Eaton. Tan circles are the shells of mummified aphids. Fuzzy brown/green portions are the entomopathogenic fungi. In between are light gray skin castings of cabbage aphids from developmental molting or deflated aphid bodies that predatory larvae leave behind.

In 2018 we observed very few aphids in the field. However, there was small preliminary experiment on the edge of the Brussels sprout field that tested the ability of netted low tunnels over Brussels sprouts plants to exclude pests. When netting was lifted to view the plants, we noticed that there were substantial infestations of cabbage aphid under one of the four replications of low tunnels. We noticed obvious fungal parasitism of aphids under this low tunnel on large infestations of aphids (Figure 11A), which was identified to likely be

Lecanicillium muscarium. The color of the fuzzy fungal mycelium sent for identification appeared brown/green on large infestations.

It is possible that environmental conditions under the low tunnel netting protected aphids but also were suitable for germination of entomopathogenic fungi. Spores of entomopathogenic fungi that attack cabbage aphid are known to germinate in the autumn during high periods of humidity (Chen et al., 2007; Reyes-Rosas et al., 2012; Shah et al., 2004). In the present experiment, it is likely that the spores germinated in the environmental conditions of 11 Oct 2018 through 13 Oct 2018 as fungi were found after this period. The average air temperature over this three-day period was 49.37°F. It did not rain during this period, however there was an average of 14 hours of leaf wetness per day. The average number of hours with ≥ 90% relative humidity per day was 18.67, and there was a low windspeed that averaged 2.53 miles per hour.

Very few aphids were observed in the field, however, a single winged aphid appeared to have been parasitized and was orange in color (Figure 11B). This singular specimen was not sent for identification.

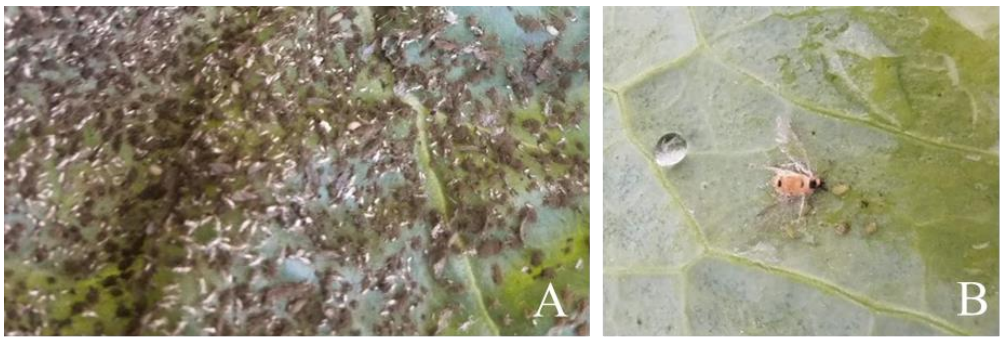


Figure 11. Entomopathogenic epizootic on cabbage aphid on Brussels sprout leaf in 2018. (A) Fungi to appear brown/green on large masses; (B) a singled winged aphid killed by fungus that appears orange in color.

DISCUSSION

We observed dramatic differences in the cabbage aphid population dynamics in the three years of this study. We hypothesize the differences in aphid populations from year to year may be due to abiotic factors, such as temperature, relative humidity, and physical knock-off of aphids through hard rain. These abiotic environmental conditions also play a role in the germination of entomopathogenic fungi. It is plausible that the crash in population at the end of 2017 decreased the overwintering of aphids (eggs or live aphids in microclimates) to continue their lifecycle into 2018. There were very few cabbage aphids in 2018. Another contributing factor may have been the substantial regular rain throughout the summer. It seems likely that rain could physically knock aphid bodies off the plant (Broadbent, 1953; Dunn and Wright, 1955). Frequent rain also increases relative humidity and literature suggests that cabbage aphid is exacerbated in drought conditions (Petherbridge and Mellor, 1936).

Azadirachtin concentration. Opender, 1998 cite that azadirachtin (from neem) applied at 30-60ppm significantly reduced reproduction of cabbage aphid in laboratory studies. In this study we evaluated two neem-based products: AzaGuard and Azera. The AzaGuard + Nu Film P treatment was applied at 75ppm azadirachtin and the Azera treatment was applied at 187ppm azadirachtin. Azera also includes pyrethrins as another active ingredient in addition to azadirachtin. Despite the application of Azera at azadirachtin concentrations over double that of AzaGuard+NuFilm P, Azera did not demonstrate efficacy against cabbage aphid that was greater than AzaGuard+NuFilm P at peak infestation on the leaves or at final harvest of sprouts. More information is needed to justify the addition of pyrethrins (such as those in Azera) to manage cabbage aphids.

Timing of application based on environmental conditions. Neem product are prone to rapid degradation (Isman, 1999) in field conditions such as sunlight, air and moisture (Henn and Weinzierl, 1989). For this reason, it may be more efficacious to apply neem products in the evening to minimize photodegradation in the first 12 hours of application. This care was not necessarily taken during this experiment and at times was applied during hot, sunny weather conditions between the hours of 10:00 AM and 4:00 PM.

Systemic properties of azadirachtin or neem. Future studies should investigate the systemic properties of azadirachtin or neem products. Neem can be applied to the roots as a dust and may keep its activity for up to 4 weeks (Henn and Weinzierl, 1989). Foliar application of neem has limited systemic action due to rapid degradation (Isman, 1999) and it is necessary to spray regularly to provide pest control. Label use of AzaGuard (azadirachtin) allows it to be applied as a drench to soil or soil-less media at a dilute rate of 0.15%-0.30%. It can also be used via chemigation with specific irrigation systems. Pavela et al., 2004 report systemic use of neem as a limited practice, however they agree that it could be used for hydroponic culture, drench treatments, or incorporation into the soil.

Stricter thresholds, spot-spraying, or plant removal. Lower economic thresholds or early spot spraying may be options for better efficacy when using foliar application of insecticides. The economic threshold could be lowered to below the current 14% used in this experiment, which may allow for earlier application of insecticides when aphid populations still are very low. Prasad et al. (2009) used a spray action threshold of 10%. Another method may be to "spot spray" or remove single leaves or plants with few aphids colonizing in the first few weeks of scouting. The frequency of insecticide application brings into question the economics of insecticide material and labor costs associated with insecticide application.

Price per insecticide application. Price range and recommended spray rates result in Azera and AzaGuard as the most expensive per application per acre of the insecticides that we evaluated. Using recommended low rates, Azera and AzaGuard + Nu Film P are approximately $65.50 and $51.00 per acre application, respectively (Table 1). Using recommended high rates, Azera and AzaGuard + Nu Film P are approximately $115.00 and $80.00 per acre application, respectively. M-pede and was the least expensive insecticide per acre application (approximately $23.00).

Effect of experimental design on results. It is important to note that in this experiment cabbage aphid insecticide plots were surrounded by untreated plots that became highly infested with cabbage aphid. In turn, this created an artificially high population of aphids compared to what most growers experience. This suggests that the chosen insecticides might have shown better efficacy under commercial farm conditions.

Naturally-occurring entomopathogenic fungus in the environment

An unexpected result of this experiment was the presence of entomopathogenic fungi on cabbage aphid. Following the peak of aphid populations on 28 Sept 2017, there was a substantial decrease in cabbage aphid populations across all treatments, including the un-sprayed control plots. We hypothesize this "crash" in population was in part due to the identified entomopathogenic fungi. This experiment demonstrated that there are at least two species of entomopathogenic fungi that attack cabbage aphid locally. In 2017 the fungus that attacked aphid colonies in our study (*Neozygites fresenii* (Entomophthorales: Neozygitaceae)) was identified to be different than the fungus that attacked colonies in 2018 (*Lecanicillium muscarium* (Hypocreales: Cordycipitareae). The same fungus, *Lecanicillium muscarium* was identified on crops in Vermont in both 2017 and 2018 (Agrin Davari, personal communication).

A strain of *Lecanicillium muscarium*, previously known as *Verticillium lecanii***,** is commercially available under the tradename Mycotal. However, this specific biological insecticide material is not currently available in the United states. Mycotrol ESO (active ingredient *Beauveria bassiana)* is an entomopathogenic fungus product that is commercially available and was used as the fifth treatment in 2018, but we were not able to assess efficacy of this product. We hypothesize that efficacy of this product in the field environment will be influenced by environmental parameters, such as: air temperature, precipitation, relative humidity, light intensity and wind speed. Increasing relative humidity commonly associated with detrimental plant-eating fungus could encourage the germination of beneficial entomopathogenic fungi and aid in the control of cabbage aphid. Increasing relative humidity can be more easily adopted in control environment agriculture, whereas field conditions of relative humidity may be difficult to control.

Throughout the season in 2017 there were cabbage aphid infestations, but for the final harvest of the sprouts there were no cabbage aphids visible from the outside view in all treatments, including the unsprayed control. We hypothesize the plummet in aphid numbers was due to an outbreak of entomopathogenic fungi and upon closer inspection, small numbers of mostly dead aphids were found on the inside of the outer leaves of the sprouts. Thus, suitability of the crop for some markets could have been compromised due to storability or physical appearance of the crop (Shah et al., 2004). We hypothesize that earlier management of cabbage aphid would reduce the ability for aphids to reach the inner part of the sprouts.

Material applications that have fungicidal properties, such as neem products (Girish and Shankara, 2008; Henn and Weinzierl, 1989) aimed to control cabbage aphid could hurt or prevent the beneficial entomopathogenic fungi. Studies combining entomopathogenic fungi with

neem products show mixed results (Castiglioni et al., 2003; Mohan et al., 2007). Azera and AzaGuard labels do not specify their compatibility with entomopathogenic fungi; further research is needed to understand the possible influences on one another.

Future investigations: different modes of action or synergists

Future studies should investigate different modes of action or active ingredients, some of which are outlined by Seaman (2016). Other university studies have confirmed poor aphid control when using M-Pede alone (Seaman, 2016). However, there has been some preliminary success using M-Pede as a synergist for various pesticides and is a topic worthy of investigation (Gilrein, personal communication). Despite moderate control of cabbage aphid using neem products, we hypothesize that using other strategies of integrated pest management could provide better control of cabbage aphid in future experiments. Systemic use of neem, targeted application times (evening application), using synergists in pesticide formulation, and conservation biological control of natural enemies are worthy of pursuing in future research.

Conclusion

In closing, cabbage aphid numbers fluctuate from year to year. Some years there are high infestations of this pest and some years economic thresholds are barely reached. On years with high infestations, growers who use organic-approved insecticides will likely gain some control of cabbage aphid using neem-based insecticides. However, further research is needed to confirm this on highly infested years without fungal epizootics that result in population crashes before final harvest. Additionally, entomopathogenic-fungi based insecticides should be evaluated for their efficacy against cabbage aphid in the field setting on years with high numbers of aphid.

CHAPTER 3:

INSECTARY PLANTS ATTRACT HOVERFLIES AND OTHER INSECTS

INTRODUCTION

Hoverflies (Diptera: Syrphidae : Syrphinae), also known as flower flies or syrphid flies, are a diverse family of insects with 6,000 described species (Mushtaq et al., 2014). Hoverflies in the subfamily Syrphinae have larvae that prey on aphids and there is potential for hoverflies in the tribes Syrphini and Melanostomini to act as biological control agents of aphids on crops (Laubertie, 2007; Haenke et al. 2009; Hickman and Wratten, 1996). Like other flies, hoverflies have two wings and can be distinguished apart from other Dipteran insects by their "false vein" (vena spuria) found in the middle of each wing between the "radius" and "media" (Laubertie, 2007). Many hoverfly adults are brightly colored, striped, and may sometimes be mistaken for bees. Characteristics that distinguish hoverflies from bees are their large eyes that take up most of their head, stubby antennae, two wings, and their aptitude for hovering (Laubertie, 2007).

The hoverflies of interest in the present study have a lifecycle that require different food sources from plants and insects (Figure 12). Adult hoverflies consume pollen and nectar, whereas the larval stages feed on insects, namely aphids (Amorós-Jiménez et al., 2014). Hoverfly adults feed from flowers for important nutrients needed for reproduction and energy (Amorós-Jiménez et al., 2014). They then use sexual reproduction and can be seen mating on flowers or flying in the air together. Subsequently, the hoverfly female then seeks a suitable place to oviposit her eggs, which usually is on a plant next to aphids, but sometimes may be next

to zero or very few aphids (Bugg et al., 2008; Chandler, 1968a, 1968b). The eggs then hatch into larvae that consume aphids (Dixon, 1977; Michaud and Belliure, 2001).

Since aphid colonies reproduce rapidly, an ideal biological control agent acts quickly while aphid populations are low. An advantage of using hoverflies as biological control agents is their ability to locate and predate on aphid colonies sooner than other predatory insects such as ladybeetles (Dixon, 2000). Hoverflies have the ability to lay eggs before any presence of the aphid (Chandler, 1968) or when populations still remain low (Chandler, 1968; Dixon, 2000). *Toxomerus marginatus* is the most prevalent species found in vegetable crops in California (Bugg et al., 2008). *T. marginatus* is more likely than other hoverfly species to lay eggs in the absence of aphids compared to other hoverfly species (Bugg et al., 2008). Tooker et al. (2006) reports *T. marginatus* and *Sphaerophoria contiqua* to be the top two prevalent aphid-eating hoverflies in central Illinois over 33 years of data collection. (Pollard, 1969) cites *Syrphus balteatus* (Degeer) and *Sphaerophoria scripta* (L.) to be the most abundant predators of cabbage aphid in England.

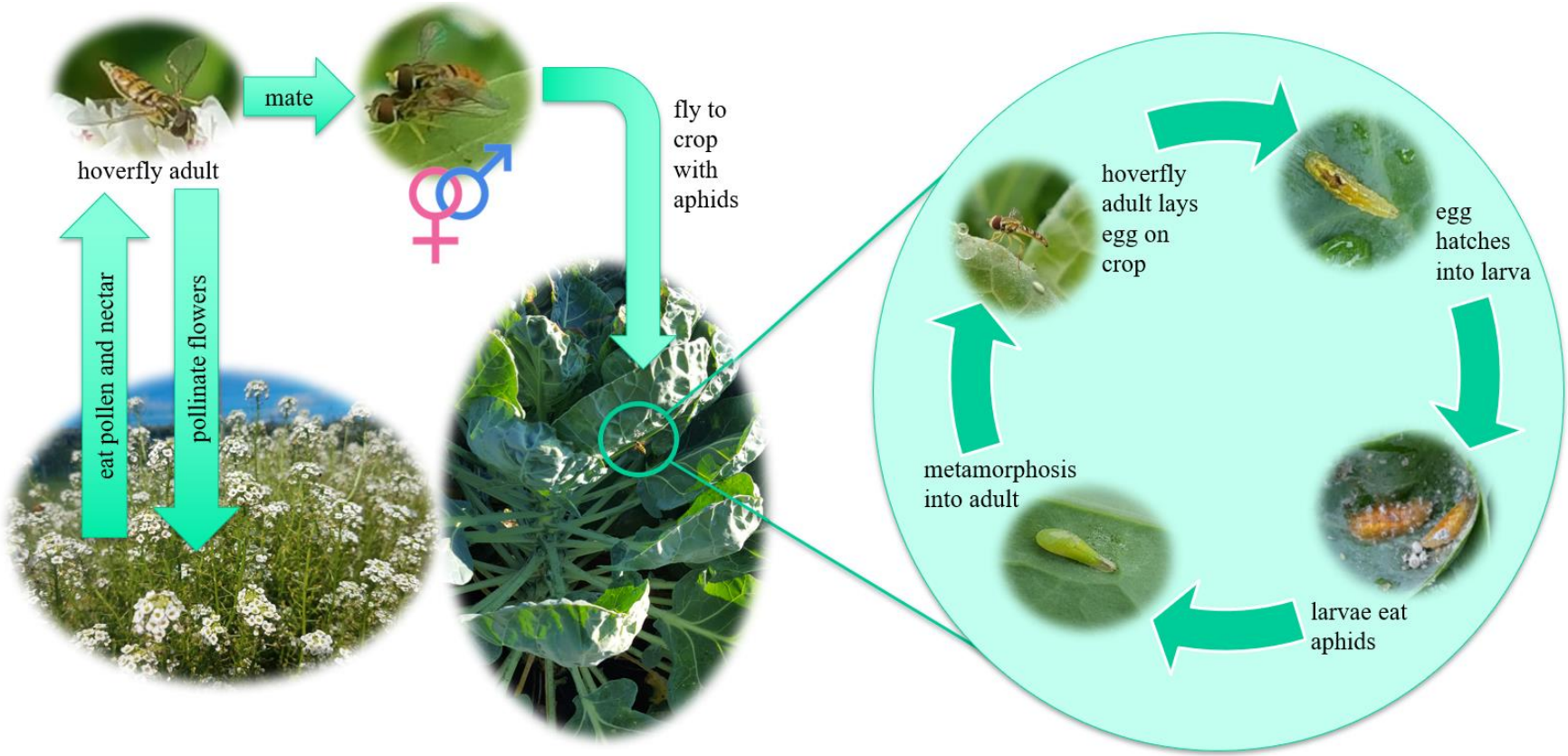


Figure 12. Lifecycle of aphidophagous hoverfly with Brussel sprout host plant. The life stage that is responsible for deleterious effects on aphids is the hoverfly larva, which then pupate and go through metamorphosis into adults.

Cabbage aphid colonies may produce winged aphids that distribute new colonies on nearby plants for a variety of reasons. Aphids that are physically stressed by the disturbance of other insects brushing against their bodies may respond by producing winged aphids. When hoverfly larvae are predating among aphid colonies they cause less disturbance to the aphids compared with other predatory insects (Michaud and Belliure, 2001). This beneficial characteristic lessens the production of winged aphids (Michaud and Belliure, 2001) and hence, distribution and spread of new aphid colonies. A field study by Hickman and Wratten (1996) that showed that predatory hoverflies can provide control of cabbage aphid. However, some studies showed limited control. Some researchers concluded poor control of aphid due to a low overall hoverfly population (Ambrosino, 2006; Tenhumberg and Poehling, 1995). Other studies cite delayed timing of gravid (egg-carrying) hoverfly females (Ambrosino et al., 2007) compared with cabbage aphid, resulting in a lack of synchronization of the pest and biological control agent (Neuville et al., 2016). In Poland, Jankowska (2005) found that although cabbage aphids were present in the field starting in the end of April, hoverfly larvae appeared in July. In England, Pollard (1969) observed cabbage aphid immigration into the field in July, whereas hoverfly larvae (*Syrphus balteatus* (Degeer) and *Sphaerophoria scripta* (L.)) were observed in early August.

Toxomerus marginatus is the most prevalent hoverfly species found in vegetable crops in California and is more likely than other hoverfly species to lay eggs next to low populations of aphids or no aphids (Bugg et al., 2008). Chandler (1968) demonstrated that three other hoverfly species (*Platycheirus peltatus* (Meig.), *Melanostoma scalare* (F.) and *M. mellinum* (L.)) chose to lay eggs on Brussels sprout plants that were free of aphids. These four species of hoverfly may be of particular interest as biological control agents, as the hatching of their larvae may allow for

better synchronization of the first aphids that colonize crop fields compared with other hoverfly species. For example, in another experiment (Chandler, 1968b) used Brussels sprouts infested with cabbage aphid and found that the hoverfly *Platycheirus manicatus* (Meig.) preferred to oviposit eggs on plants with about 100 aphids per plant. Whereas, in the same study *Platycheirus scutatus* (Meig.) preferred ovipositing on plants with about 1000 aphids and *Syrphus ribesii* (L.) preferred ovipositing on plants with about 2000 aphids per plant. They concluded that timing of oviposition in relation to size and distribution of cabbage aphid colonies is dependent on hoverfly species.

Identifying the species of hoverflies present in a field setting is a meaningful piece of information to understand their ability to accomplish biological control. Specifically *T. marginatus* may be a potential biological control agent in this region, but this has yet to be demonstrated in the literature. Tooker et al. (2006) reports *T. marginatus* and *Sphaerophoria contiqua* to be the top two prevalent aphid-eating hoverflies in central Illinois over 33 years of data collection. Pollard (1969) cites *Syrphus balteatus* (Degeer) and *Sphaerophoria scripta* (L.) to be the most abundant predators of cabbage aphid in England.

Using insectary plants to augment natural enemies of cabbage aphid

"Classic" biological control and "inundative release" both introduce insect enemies into an area to control pests (Laubertie, 2007). In contrast, this experiment focuses on "conservation" biological control by manipulating the agroecosystem to attract and enhance natural enemies already present in the region (Debach and Rosen, 1991). While hoverflies have been observed feeding a variety of weeds in the field, (Cowgill et al., 1993; Hickman et al., 1995a) food sources for adult hoverflies near cash crops can be limited because modern agricultural production tends to eliminate flowering weeds near cash crops (Boatman, 1989). A way of encouraging biological

control is through provision of essential requirements (Bacci et al., 2009) such as food or habitat. The use of insectary plants, or plants that attract beneficial insects, may potentially be an economically practical way of encouraging and augmenting natural predation of aphids by hoverflies that are already present in the environment. Insectary plants provide hoverflies with food sources that are required for reproduction (Amorós-Jiménez et al., 2014). Subsequently, these adult insects fly to the neighboring crop plants (i.e. Brussels sprouts) and lay eggs that hatch into progeny that prey on aphids. Production of hoverfly eggs is the main driver of biological control, since the eggs give rise to predatory larvae that consume live aphids.

Flowers provide adult hoverflies with nectar as an energy source (Laubertie et al, 2012) and pollen provides protein needed for sexual maturation and egg development (Amorós-Jiménez et al., 2014; Hickman and Wratten, 1996; Rodríguez-Gasol et al., 2019). Several studies have shown that insectary plantings encourage adult hoverflies to stay within the crop field longer which increases aphid predation rates and reduces crop damage (Amorós-Jiménez et al., 2014; Haenke et al., 2009; Hickman and Wratten, 1996; Pineda and Marcos-García, 2008). In some cases, hoverfly larvae provided an economically meaningful control of aphids (Hickman and Wratten, 1996) or provided a measurable decrease in populations (White et al., 1995).

Hogg et al. (2011) describes two independent mechanisms in which insectary plants may contribute to pest suppression by hoverflies. First, insectary plants may promote a higher predation rate simply because adult hoverflies are initially attracted to the crop field by the insectary plants and stay in the area (Amorós-Jiménez et al., 2014; Haenke et al., 2009; Hickman and Wratten, 1996; Hogg et al., 2011a; Lövei et al., 1992; Molthan and Ruppert, 1988; Pineda and Marcos-García, 2008; Ruppert and Molthan, 1991; Sengonca and Frings, 1988). Harwood et al. (1994) describes this as increased "hoverfly local density." Second, the quantity of available

nectar and pollen has been demonstrated to have a significant effect on the amount of eggs produced by hoverfly females (Hogg et al., 2011; Scholz and Poehling, 2000). Furthermore, Harwood et al. (1994) describe a third mechanism: when flowering plants are available, it may lead to an increase in hoverfly species diversity near crops.

Drawing the distinct connection between provision of floral resources and aphid suppression in the field setting is difficult (Hogg et al., 2011). It is also important to note that when publications report significant "decreases" in aphid numbers, it does not mean that full economic "control" of the pest was obtained. Control can be defined as the full elimination of cabbage aphid, or the presence of some aphids that do not affect harvest size, storability or marketability of the crop. Some studies report control of aphids from hoverfly larvae (Brennan, 2013; Brennan, 2016; Hickman and Wratten 1996), whereas other experiments only demonstrated significant decreases in aphid populations (Chambers and Adams, 1986; White et al., 1995). Hogg et al. (2011a) implemented a caged field experiment to study biological control of aphids on lettuce. They evaluated hoverfly fitness with access to no pollen (water only), nectar, or alyssum flowers (*Lobularia maritima* (L.) Desv. (Brassicaceae)) that provided both nectar and pollen. Provision of alyssum resulted in significantly enhanced egg production, greater populations of hoverfly larvae and fewer aphids than the control and nectar treatments. Ambrosino et al. (2007) reported limited control of aphids and attributed it to the delayed appearance of gravid (egg-carrying) female hoverflies. Other researchers concluded poor aphid control was due to a low overall hoverfly population (Ambrosino, 2006; Tenhumberg and Poehling, 1995).

Beyond aphids, hoverfly larvae have been known to predate on other *Brassica* pests. Miller (1918) reports the two most abundant hoverflies in New Zealand (*Syrphus novae-*

zealandiae and *Melanostoma fasciatum*) predate on diamondback moth caterpillars (*Plutella xylostella* L.), a lepidopteran pest of *Brassicas* (Miller, 1918). Hoverfly larvae are also known to predate on imported cabbageworm moth (*Pieris rapae*), another lepidopteran pest of *Brassica* crops (Ashby and Pottinger, 1974). These findings led Laubertie (2007) to the conclusion that the conservation of beneficial insects through insectary plantings can contribute to the management of not only cabbage aphid, but several other pest species as well.

Insectary plant species selection

Weeds, perennial hedgerows (Cowgill et al., 1993; Gilbert, 1981; Hickman et al., 1995a) and herbs have been surveyed for adult hoverfly attractiveness and preference in other field experiments. The present study focuses on insect preference of annual flowering plants that are commonly used in conservation biological control and can be seed-propagated on the farm.

Plant species used in this experiment were **alyssum** (*Lobularia maritima*), **buckwheat** (*Fagopyrum esculentum* Moench), **cilantro** (*Coriander sativum* L.), **dill** (*Anethum graveolens* L.), **phacelia** (*Phacelia tanacetifolia)*, **fennel** (*Foeniculum vulgare*) and **calendula** (*Calendula officinalis* L.) It is important to note that common names such as "marigold," and "pot marigold" may be used to describe *Calendula officinalis* L. The seven flowering plant species (Table 3 and Table 4) were chosen as insectary plants for this study based on three criteria: (1) previous reported success in attracting natural predators and parasitoids of aphids in other studies (Brennan, 2016; Hickman et al., 1995); (2) availability of seed to local growers; (3) plants with short days to maturity. Plants with short days to maturity are likely to bloom during or before July. A continuous bloom is important because flower nectar supports beneficial insect fecundity (the ability to reproduce) and in turn, rate of aphid predation. Care was taken to select

cultivars and species for insectary plants that were readily available to growers by purchasing from local suppliers (Johnny's Selected Seeds, Albion ME; Fedco Seeds, Clinton, ME).

Alyssum (Brennan, 2013; Colley and Luna, 2000), **phacelia** (Harwood et al., 1994; Hickman et al., 1995b, 1993; Laubertie, 2007; Wratten et al., 2003) **coriander/cilantro** (Colley and Luna, 2000; Hickman et al., 1995b; Laubertie, 2007, 2007; Wratten et al., 2003), **buckwhea**t (MacLeod, 1992; Laubertie, 2007; Lövei et al., 1993; Colley and Luna, 2000; Wratten et al., 2003; Hickman et al, 1995) and **calendula** (Colley and Luna, 2000; MacLeod, 1992) have been previously evaluated as insectary plants for attractiveness and increased fitness in hoverflies (MacLeod, 1992; Hickman et al., 1995; Colley and Luna, 2000; Wratten et al., 2003).

In the United Kingdom, MacLeod (1992) evaluated buckwheat, cilantro, sunflower (*Helianthus annuus* L.), borage (*Echium lycopis* L.), and calendula for their attractiveness to hoverflies. MacLeod (1992) found cilantro and buckwheat to be the most attractive plant species to hoverflies. Colley and Luna (2000) also investigated the relative attractiveness of insectary plants to hoverflies in a field setting and found similar results. The annual crops evaluated by Colley and Luna (2000) were buckwheat, phacelia, cilantro, mustard, alyssum, calendula, and marigold (*Tagetes patula* L.). Colley and Luna (2000) concluded that cilantro had the greatest potential for hoverfly attraction, but also deemed alyssum, phacelia, buckwheat, mustard, and fennel as "significant providers of floral resources for hoverflies."

Table 3. Insectary plant days to maturity, seeding, and transplanting dates, grown in Durham, NH in 2017. Days to maturity data were provided by seed suppliers. Colored blocks show bloom period throughout the growing season. Numbers following plant species indicate succession plantings sown on different dates. Plants still flowering in November were terminated by frost.

Treatment	Cultivar name	Plants per plot	Days to maturity	Seed into trays date	Transplant date	Jul	Aug	Sept	Oct	Nov
Alyssum	Sweet alyssum	45	50-60	20-Jun	11-Jul					
Buck-wheat 1	Unnamed	27 oz seed	35-42	20-Jun	29-Jun					
Buck-wheat 2				21-Jul	direct seed					
Cilantro 1	Santo	45	55 leaf, 90-105 seed	20-Jun	11-Jul					
Cilantro 2				20-Jul	11-Aug					
Dill 1	Bouquet	45	55 leaf, 85-105 seed	20-Jun	11-Jul					
Dill 2				20-Jul	11-Aug					
Phacelia	Unnamed	30	60-70	20-Jun	11-Jul					
Fennel	Grosfru-chtiger	30	50-60 leaf	20-Jun	11-Jul			never flowered		

Table 4. Insectary plant days to maturity, seeding, and transplanting dates, grown in Durham, NH in 2018. Days to maturity were provided by seed suppliers. Colored blocks show bloom period throughout the growing season. Numbers following plant species indicate succession plantings sown on different dates. Plants still flowering in November were terminated by frost.

Treatment	Cultivar name	Plants per plot	Days to maturity	Seed into trays date	Transplant date	Jul	Aug	Sept	Oct	Nov
Alyssum	Sweet alyssum	45	50-60	31-May	20-Jun					
Buckwheat 1					21-Jun					
Buckwheat 2	Unnamed	27 oz seed	35-42	direct seeded	4-Jul					
Buckwheat 3					2-Aug					
Cilantro 1	Santo	45	55 leaf, 90-105 seed	31-May	28-Jun					
Cilantro 2				1-Jul	1-Aug					
Dill 1	Bouquet	45	55 leaf, 85-105 seed	31-May	20-Jun					
Dill 2				1-Jul	1-Aug					
Calendula 1	Alpha (organic)	30	50-55	7-Jun	25-Jun					
Calendula 2				3-Aug	9-Aug					

Attributes of flowers

Several factors, including flower color, shape, nutritional value and odor appear to affect hoverfly attraction or feeding preferences (Gilbert, 1981; Haslett, 1989; Hickman et al., 1995b; Laubertie, 2007).

Flower color. Cowgill, (1989) reports the colors white and yellow induce feeding in hoverflies and other studies demonstrate aphid-eating hoverflies prefer the color yellow (Hoback et al., 1999; Kevan and Baker, 1983, 1983; Laubertie, 2007). The present study includes alyssum, buckwheat, dill, and cilantro, which all display white and/or yellow coloring in their flowers. However, Haslett (1989) criticizes the use of color descriptions as seen by the human eye compared to the insect visual spectrum. Haslett (1989) argues we can avoid misinterpretation of color by using reflectance spectra through use of a reflectance spectrophotometer. Haslett (1989) evaluated hoverfly preferences and found different hoverfly species to be attracted to an array of reflectance spectra colors (yellow, blue and violet, white).

Flower morphology. Flower morphology affects insect access to nectar (Jervis et al., 1993). Pollen and nectar are often hidden or difficult to access, depending on morphology and behavior of the insect visitor as well as the architecture of the flower (Wäckers and van Rijn, 2012). The head width and mouthpart length of the insect visitor must match the flower measurements and openings that allow access to nectar and pollen (Wäckers and van Rijn, 2012). Umbelliferous (Apiaceae) flowers have a short corolla which may allow hoverflies to access nectar (Gilbert, 1981; Wäckers and van Rijn, 2012) and are therefore ideal insectary plants for hoverflies. The present study includes cilantro, dill, and fennel as umbelliferous flowering plants. Baggen et al. (1999) reports the corolla depth of dill to be zero, meaning that there is full access to the nectaries. Wäckers and van Rijn, (2012) agree that the Apiaceae family (*Ammi*

majus, cilantro, and fennel) and Polygonaceae family (buckwheat) have fully exposed nectaries and categorize these specific plants as having <0.2mm floral nectar depth.

In contrast, the shape of some flowers may not allow for the extraction of nectar by hoverflies. Wäckers and van Rijn (2012) conclude that zoophagous (insect-eating) hoverflies can reach nectar at a depth that is less than 2.0mm. In a later report, Wäckers and van Rijn, (2016) found the proportion of zoophagous hoverfly observed on insectary plants showed a stepwise decline when the depth of corolla is greater than 1.6mm. White et al. (1995) report that the long corolla of phacelia and short mouth parts of hoverflies do not allow hoverflies to gain access to nectar but may still be a pollen source. Baggen et al. (1999) reported similar findings and emphasize the need for hoverflies to access the nectaries of the flowers. They found the diameter of the phacelia corolla to be relatively large (5.05mm), however there are only 0.15mm openings for access due to stamen blocking the entrance. They classified phacelia to have a floral nectar depth of more than 3.0mm, however, they cite phacelia to be an exception to the 2.0mm rule. Since a specific hoverfly (*E. balteatus*) was used as a model organism for much of their literature analysis, we hypothesize that the ability for phacelia to be a suitable food source may depend on the morphology and behavior of the hoverflies present.

Flower odor. Laubertie (2007) investigated the effect of odor in hoverfly attraction using yellow water traps to capture hoverfly species. Traps with rose-water odor caught significantly more adult hoverflies (*Melanostoma fasciatum* (Macquart)) than control water traps without odor. They found an interaction between rose-water traps and species, whereas there was no interaction between control water traps and species. Rose-water traps were attractive to both male and female *M. fasciatum,* whereas rose-water traps were attractive to only female *Melangyna novaezelandiae* (Macquart)). Furthermore, fewer *M. novaezelandiae* males were

collected from rose-water traps than the control water traps. Another study by Molleman et al. (1997) evaluated odor as an attractant to hoverflies using methyl salicylate, which is a synomone isolated from the volatiles of pear trees released after psylla pests feeding on leaves. Their results showed that methyl salicylate was attractive to some hoverfly species (*Episyrphus auricollis* (Meigen), *Metasyrphus luniger* (Meigen), *Metasyrphus corollae* and the '*Syrphus ribesii* (L.)' complex) but not to another species (*Episyrphus balteatus*).

Flower nutrition to hoverflies: impact on fitness and reproduction

Net reproduction rate. Laubertie et al. (2012) compared insectary flower diets for their effect on net reproduction rate (mean number of female eggs produced by females during their lifetime), using 5 of the 7 species evaluated in this study using *E. balteatus* as a model organism. They ranked the net reproduction rate as: phacelia (349) > buckwheat (290) > cilantro (221) > alyssum (64) > mustard (27) > calendula (10). This suggests that it may be important to consider not only attractiveness, but also the nutritional value of floral resources.

Alyssum. Laubertie et al. (2012) studied the hoverfly species *E. balteatus* and found that although female hoverflies fed a diet of alyssum had longevity (lived a long time), only a small proportion laid eggs successfully. However, even if alyssum flowers were less nutritious to hoverflies than other insectary plants, Hogg et al. (2011a) concluded that without access to alyssum as a food source, *Eupeodes fumipennis* eggs either did not develop or were resorbed back into the body of the adult hoverfly.

Buckwheat. Wäckers and van Rijn (2010) found buckwheat to be a suitable food source; *E. balteatus* hoverfly adults survived on this food source for six days and could reproduce. Wäckers and van Rijn (2010) also report buckwheat as one of ten plant species on which *E. balteatus* had longevity equivalent to their sucrose (positive control) treatment. Provision of 1-M

sucrose positive control allowed for an average lifespan of 11.3 days for *E. balteatus*, whereas provision of buckwheat allowed for an average of 13.5 days and 12.4 days for female and male adults, respectively. Buckwheat resulted in the highest adult survival time, above cilantro, fennel, phacelia and calendula.

Cilantro. Wäckers and van Rijn (2012) found cilantro to be a suitable food source; *E. balteatus* hoverfly adults survived on this food source for six days and could reproduce.

Dill. Literature does not show explicit or apparent information regarding the effect of dill floral resources on hoverfly fitness. However, Wäckers and van Rijn (2012) conclude that flower choice and number of hoverflies in the field setting correlate well with nectar accessibility and adult survival, and the flower architecture of dill allows for nectar accessibility.

Fennel. van Rijn and Wäckers (2016) found that hoverflies on a diet of fennel flowers resulted in longer adult survival measured in days (8.4 female, 8.2 male) compared with phacelia (4.0 female, 3.6 male) and calendula (2.3 female, and 2.9 male). In a previously written article Wäckers and van Rijn (2012) define insectary plants to have adequate nutrition when hoverflies survived for six days to reproduce. However, van Rijn and Wäckers (2016) did not measure reproductive success. Since longevity is not always linked with reproductive success (Laubertie et al, 2012) that is necessary for biological control, more studies are needed on the link between fennel floral nutrition and adult hoverfly reproduction.

Phacelia. Provision of phacelia flowers to hoverflies has shown mixed results in both field and laboratory studies. van Rijn and Wäckers (2016) found that hoverflies on a diet of phacelia flowers resulted in a shorter adult survival (days) than cilantro, fennel, and buckwheat. Provision of phacelia flowers only resulted in a greater lifespan than calendula compared with the plant species discussed in the present study. However, since the lifespan of these species of

hoverflies on these diets were so short, it is unlikely that there was successful reproduction that is necessary for the goal of biological control of aphids. Other studies produced conflicting results.

Hickman and Wratten (1996) described an increase in hoverfly oviposition within 100 meters of phacelia (Hickman and Wratten, 1996) and confirmed that phacelia is found abundantly in the gut of gravid females (Hickman et al., 1995a) on the North Island of New Zealand. Also, in New Zealand, White et al. (1995) demonstrated that phacelia strips sown around the edges of cabbage increased hoverflies (*Melanostoma fasciatum (Macquart)* and *Melangyna novaezelandiae* (Macquart)) and decreased aphids. However, there are many species of hoverflies throughout the world, and *Melangyna novaezelandiae* (Macquart) is endemic (Laubertie, Wratten, and Sedcole, 2006). The hoverflies identified in our region represent different species and therefore different physiology and behavior from those identified by the aforementioned New Zealand studies.

Laubertie et al. (2012) found phacelia to have the highest net reproductive rate compared to four other insectary plant treatments in this present study. Laubertie et al. (2012) conclude that since nectar is largely excluded by the architecture of the reproductive parts of phacelia, pollen quality and/or abundance is key in understanding hoverfly nutrition and its implications in biological control. However, it is very important to not draw conclusions about zoophagous hoverflies as a whole, since *E. balteatus* has been categorized as a pollen feeder, or pollen specialist (Branquart and Hemptinne, 2000; Gilbert and Owen, 1990).

However, nectar plays the role of giving hoverfly adults energy. Since this energy source is likely lacking in phacelia, it potentially could be replaced with "honeydew", a secretion of aphids. For aphids, honeydew may be less nutritious than nectar, as has been reported for parasitoids (Lee et al., 2004) but it has been shown to enhance survival of *E. balteatus* (van Rijn

et al., 2006). In a later experiment, van Rijn and Wäckers (2016) demonstrate that nectar –not pollen—accessibility is key to deciding flower choice and abundance of insect-eating hoverflies in field experiments.

Calendula. van Rijn and Wäckers (2016) report most plant species nutritionally support adult hoverflies to live longer as compared with solely providing hoverflies with water. However, calendula was one of the exceptions; calendula provides very little benefit to *E. balteatus* hoverfly fitness in their study (van Rijn and Wäckers, 2016). Net reproduction rate was extremely low on *E. balteatus* fed a calendula flower diet; it was significantly lower than phacelia, buckwheat, cilantro and alyssum.

Goals, objectives and hypotheses

This study of the relationship between insectary plants and hoverflies takes a survey approach in a field setting with the purpose of creating a foundation for narrowing experimental design for future researchers. Our goal is to understand how insectary plants are used by hoverfly species in our region and investigate this augmentative biological control strategy to decrease populations of cabbage aphid. Specific objectives of this experiment were to:
(1) Quantify and describe the duration of bloom of seven insectary plant species from July until frost; **(2)** Quantify the number of hoverflies observed on different plant species and test for differences; **(3)** Quantify the number of other insects observed on different plant species and test for differences; **(4)** Identify hoverfly species collected from insectary plants. For objectives (2) and (3) we hypothesized that different species of insectary plants would attract different numbers of hoverflies and other insects.

MATERIALS AND METHODS

Experimental design. The insectary plants were transplanted or direct-sown in a randomized complete block design with 4 replications. Succession plantings were used for plants with short days to maturity to maximize the bloom time and to increase our ability to evaluate differences of hoverfly densities throughout the growing season. For this experiment, the same cultivar (i.e. 'Santo' cilantro) was seeded on two different dates (i.e. cilantro 1 and cilantro 2), with the goal of a continuous bloom period for beneficial insect forage. The first succession is notated with "1" (i.e. cilantro 1) and the second succession is written "2" (i.e. cilantro 2). For insectary plants with a continuous flowering habit (i.e. alyssum, phacelia) only one planting date was used and therefore no succession number was assigned. In 2017 there were 9 treatments, which included: alyssum, buckwheat 1, buckwheat 2, cilantro 1, cilantro 2, dill 1, dill 2, phacelia and fennel (Table 3). In 2018 the same treatments were used, except phacelia did not have a high enough germination rate to be included and fennel was omitted in 2018 since it did not flower in 2017. Instead, phacelia and fennel were replaced with calendula 1 and calendula 2. Buckwheat 3 was also added for a total of 10 treatments (Table 4).

The randomized complete block design of insectary plants was approximately 68 feet x 18 feet, totaling 1224 square feet dedicated to insectary plants and spaces in between plots. Each plot was a three-foot-wide raised bed by 5 feet long for a total of 15 square feet per plot. This randomized complete block design was in the corner of a Brussels sprout crop field in both years and served as a "refuge" insectary planting (Figure 13 and Figure 14). Since alyssum has been shown to increase beneficial insect fitness in the literature (Hogg et al., 2011), it was included as a treatment in the randomized complete block design, and additionally 50% of the insectary plant

area was dedicated to a large swath of alyssum that directly abutted the Brussels sprouts planting. This swath of alyssum was used for other experiments and not directly used for the experiment described in this chapter (Figure 15 and Figure 16).

Figure 13. Photo of field in 2017. The field is bordered by grapes on the left and insectary plants are abutted by Brussels sprouts plants in the middle background. Beyond the Brussels sprouts in the background there are low tunnel strawberries (white).

Figure 14. Photo of field in 2018. The field is bordered by grapes on the left, abutted by Brussels sprouts plants in the middle background, and abutted by strawberries in low tunnels on the right. The plot in the front shows a succession planting recently transplanted into a field with already blooming insectary plants.

Figure 15. Photo of additional alyssum planting in 2017. In addition to the randomized complete block design of insectary plants the alyssum planting in the middle of the photo comprises 50% of the area devoted to insectary plants and was used for a different experiment but may have affected hoverfly visitation to the randomized complete block design of insectary plants. This alyssum planting directly abuts other insectary plants (on the left) and Brussels sprouts plants (on the right).

Figure 16. Photo of additional alyssum planting in 2018. In addition to the randomized complete block design of insectary plants this alyssum planting comprises 50% of the area devoted to insectary plants and was used for a different experiment but may have affected hoverfly visitation to the randomized complete block design of insectary plants. This alyssum planting directly abuts other insectary plants and Brussels sprouts plants (in the background).

Insectary plant seedling production. Most seeds of insectary plants were sown into 128-cell trays using Promix BX (Pro-Mix, Quakertown, PA) soilless media and grown in a high tunnel until transplant at Woodman Farm in Durham, NH (Table 3 and Table 4). Seedling trays were fertilized with water soluble fertilizer two times before transplant [15N–2.2P–12.5K] (Peters Professional 15–5–15 Cal–Mag; Everris Intl., Geldermalsen, The Netherlands) at a rate of 300 ppm N. In 2017, buckwheat was direct seeded in the field and seeded in the greenhouse (2 seeds per cell) for filling gaps in the field where direct seeding survival was patchy. In 2018, both direct seeding and tray-seeded phacelia germination rates did not yield enough plants for a replicated treatment.

Field site preparation. Field experiments were conducted in the same field in 2017 and 2018 at the University of New Hampshire at Woodman Horticultural Farm in Durham, New Hampshire, United States (lat. 43.150591ºN, long. 70.942150º). Prior to transplanting into the field, 150lbs/acre of nitrogen (N) as 27-0-0 was incorporated in the spring based on soil test recommendations. Six-foot spaced beds were created with 1 line of drip tape buried 1 inch below the soil surface. Raised beds were covered with 0.6 mil Organix A.G. Film biodegradable black plastic mulch (Organix Solutions, Phoenix, Arizona) and slits were cut into the plastic mulch to seed and transplant insectary plant treatments. Seedlings of alyssum, cilantro 1, dill 1, fennel, and phacelia were transplanted at 20 days of maturity on 11 July 2017 and 20 June 2018, except for 2018 when phacelia and fennel were omitted. Seedlings of cilantro 2, dill 2, and calendula 2 were transplanted at 30 days of maturity on 11 Aug 2017 and 1 Aug 2018. Buckwheat was direct seeded 20 Jun 2017 and backup transplants were used to fill in gaps of the first planting on 29 Jun 2017. The seeding rate was then increased to 27 oz of seed per plot to ensure adequate plant density for the subsequent plantings in 21 Jul 2017, 21 June 2018, 4 Jul 2018, and 2 Aug

2018 and no backup transplants were needed. Seedling density per insectary plant treatment in 2017 and 2018 are outlined in Table 3 and Table 4.

Irrigation. The timing of irrigation events was determined by regular evaluation of the root zone. A clump of soil and the squeeze test was used to determine soil moisture (Healy, 2012). If a ball or clump of soil could be formed, no water was added. If the soil was loose and falling apart, the drip irrigation was turned on for an hour interval and the root zone was re-evaluated for moisture. Throughout the course of the season bio-degradable mulch did start to rip and fray but did not seem to negatively affect the insectary plants. The bare soil exposed by rips needed more irrigation during sunny weather, but during rainy and moist periods allowed the overhead water to penetrate the soil bed. On average, the drip irrigation ran about 1.5 hours weekly in 2017, whereas in 2018 drip irrigation was only run twice due to regular rainfall.

Data collection. Plots were observed weekly to collect first bloom and last bloom data for each treatment. All four replicates had to be flowering to consider a treatment to be flowering.

In order to test insect densities over time, observations were taken on days that were not raining with less than five miles an hour of wind and only treatments with open flowers in all four replicates were observed for number of insects. Insect density was measured by placing a 20-inch diameter (314 in^2) plastic ring over a flowering plot (Figure 17). Each plot was observed for two minutes with the naked eye. The number of insects that flew inside the ring or were already present inside of the ring were counted and recorded in a field notebook. The ring was then moved within the same plot to a different location and was observed again for two minutes and then summed per plot for a total of a four-minutes per plot. Insect density is described as the number of insects/314 in^2/4 min. Insects were counted if they flew through the ring during the

given time frame; they did not need to land or feed to be counted. Insects that fed from different clusters of flowers within the ring were counted as once. Insects that left the ring and re-entered again were counted as an additional insect observed as described by Colley and Luna (2000). Observations were taken before noon as described by Gilbert (1985) based on his observation that 10:00 AM to 12:00 PM has the highest hoverfly activity in the United Kingdom. Our focus was on hoverflies (Diptera: Syrphidae), but we also observed a suite of other insects.

Figure 17. Flowering alyssum with 20-inch diameter (314 in^2) black plastic ring for timed insect observations. The ring was placed on a plot and insects were observed for 2 minutes. The ring was then moved to another location in the plot and observed for another two minutes. For each insectary plant, insects were observed during a four-minute period in each of four replicates.

In 2017 we counted number of hoverflies, other flies (from the order Diptera, not including Syrphidae), honeybees (Hymenoptera: Apidae: Apini: Apis), bumble bees (Hymentoptera: Apidae: Bombus), other bees (Hymenoptera: Apoidea), other large hymenoptera (all insects from the order hymenoptera that are not bumble bees, honeybees, or bees and approximately ≥1cm), lady beetles adults and larvae (Coleoptera: Coccinellidae), ants

(Hymenoptera: Formicidae), fleabeetles (Coleoptera: Chrysomelidae) spiders (Araneae). Since insect visitors were numerous and challenging to count when moving in different directions, we decided to limit the groups of insects that we counted in 2018 to allow for more precise insect counts.

In 2018 we counted hoverflies, honeybees, bees, bumble bees and lady beetles. Additionally, we counted observations of imported cabbageworm adult moths (Lepidoptera: Pieridae: *Pieris rapae*), since their larval caterpillar stage is a common *Brassica* pest.

Statistical analysis. Using JMP Pro statistical software, an ANOVA was conducted to compare least-squared means of hoverfly observed per four minutes for each observation date and also for the whole season (cumulative) to determine the effects of insectary plant type insect density. Tukey's honestly significant differences (HSD) and Students T-test (when there were only two factors to compare) were used to calculate means separation at α=0.05.

Sweepnet hoverfly specimen. We used sweepnet samples to confirm identification of hoverfly species present on the farm. On mornings free of rain with less than 5 miles per hour of wind, a standard 15-inch mesh insect net was used to collect hoverfly specimens. Two passes were made with the sweep net, lightly grazing the top of the insectary flowers. Each sweep covered approximately a 180º arc across the length of each plot in each direction (Figure 18). Hoverflies were terminated with an ethanol squirt bottle in the field and then stored in ethanol. The four replicates of specimens were pooled per treatment in a single vial per date. Specimens were then dried, pinned, labeled, and organized by visual species type and samples were sent to the Canadian National Collection of Insects in Ottowa, Ontario for official identification under

the supervision of Michelle Locke. Identified specimens were compared with specimens from the University of Massachusetts to look for overlapping hoverfly species and trends.

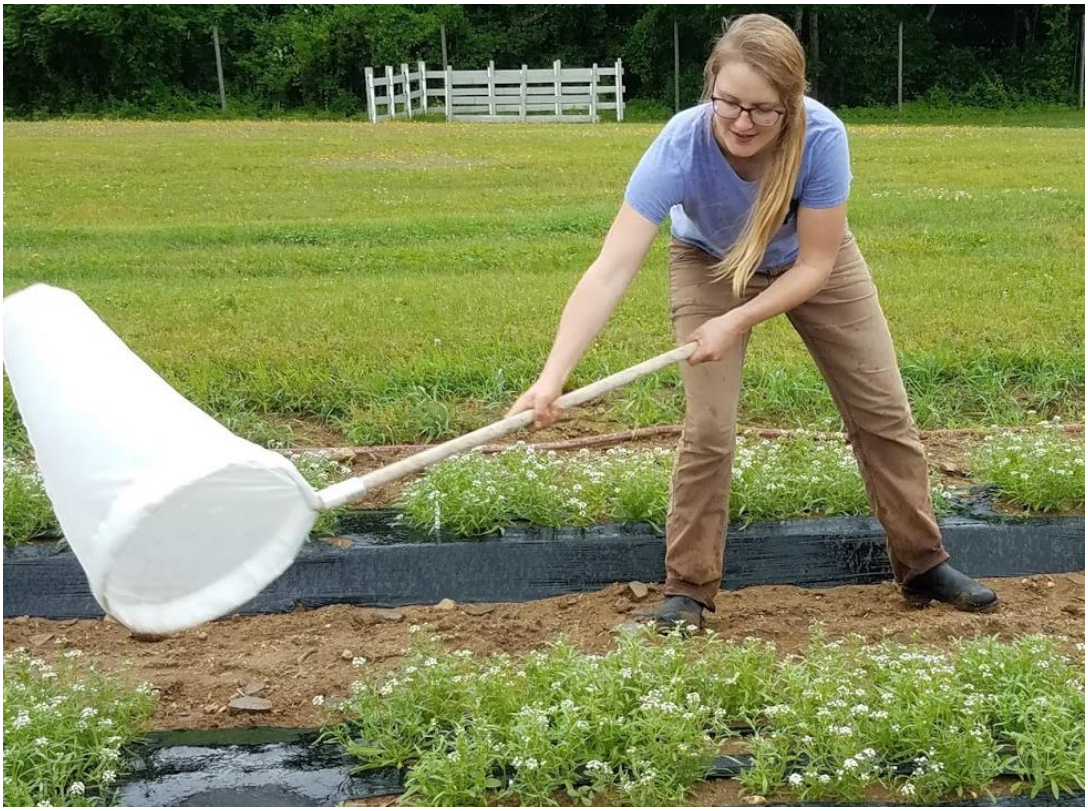


Figure 18. Example of a sweepnet lightly grazing the top of a young alyssum planting in 2019.

RESULTS

Insectary plant bloom period

Bloom period 2017. The planting dates allowed for a continuous bloom of at least one flowering species from July until frost. The bloom period began with buckwheat 1 at the end of July and ended after hard frosts in November with alyssum (Table 3).

The bloom period for buckwheat 1 and buckwheat 2 was about one-month long. In contrast, alyssum flowered from 20 Jul 2017 until after the first visible frost (18 Oct 2017) until mid-November. Buckwheat 1 completed its bloom period when alyssum was only roughly half-way through their bloom period (Table 3). Cilantro 2 and dill 2 just barely started blooming on 27 Oct 2017 before they were killed by the frost. Fennel never flowered. Phacelia bloomed from the end of August until frost.

Bloom period 2018. Bloom periods were consistent with 2017. The bloom period of all plant species started on 20 July 2018 with buckwheat 1 and ended at the frost in November with alyssum, cilantro 2, dill 2, calendula 1 and calendula 2 still flowering (Table 4). Calendula 1 continued to flower until hard frost; it was noted that a second succession would not be necessary, although the first flowers of calendula 2 were noticeably larger than calendula 1. Cilantro 2 and dill 2 trans-planting dates were moved 10 days earlier in the year to 1 Aug 2018, and both started blooming on 5 Oct 2018.

Effect of flower density and hoverfly density over time

There were significant differences between alyssum flower density throughout the season (Table 5). On the first two observation dates (26 Jul 2017 and 2 Aug 2017) the number of

alyssum flowers per 314 in^2 were significantly fewer than the remaining dates tested. The following two dates (10 Aug 2017 and 17 Aug 2017) had significantly greater flower densities than all other dates tested. By 5 Sept 2017 the flower density of alyssum was intermediate compared to the preceding dates. For the last three dates (19 Sep 2017, 27 Sep 2017, and 23 Oct 2017) data were not replicated and therefore statistical differences were not tested. The data of hoverfly density were not statistically significant at $\alpha = 0.05$. With a p-value of 0.09, there were no differences between number of hoverflies per 314 in^2 observed on alyssum tested against itself (Table 5).

Table 5. Mean number of alyssum flowers and hoverflies per 314 in^2 per four minutes observed on alyssum throughout the 2017 season.

Date	Number of flowers	Number of hoverflies
26 Jul 2017	27.9 c[z]	1.3
2 Aug 2017	191.1 c	3.7
10 Aug 2017	1739.8 a	10.0
17 Aug 2017	1834.8 a	5.0
5 Sep 2017	776.6 b	0.0
19 Sep 2017	1207.8[y]	24.3
27 Sep 2017	1360.8[y]	5.5
23 Oct 2017	1792.5[y]	1.8
p	<.0001	0.0948
F	54.7	2.5
Df	4, 12	4, 12
SEM	131.3	2.4

[z]Within column, means followed by the same letter are not significantly different according to Tukey's HSD at $\alpha = 0.05$.
[y]The last three dates of number of flower data did not have replication and therefore significant differences were not tested on these dates in both columns. *p*, F, df and SEM values do not pertain to these dates.

Combined hoverfly density 2017-2018

Combined between 2017 and 2018 the number of hoverflies observed in alyssum plots was greater than or not significantly different from other insectary plants on 11 out of 19 dates, buckwheat on 6 out of 11 dates, cilantro on 6 out of 15, and dill on 3 out of 12 dates (Table 6 and Table 8). In 2017 the number of hoverflies observed in phacelia plots was minimal compared to other treatments and zero out of 4 flowering dates had hoverfly density that was greater than or not significantly different than other insectary plants (Table 6). In 2018 the number of hoverflies observed in calendula plots was low and zero of the 11 flowering dates had hoverfly density greater than or not significantly different from other insectary plants (Table 8).

Effect of insectary plants on hoverfly density over time: comparison by date, 2017

We evaluated adult hoverfly density on all actively flowering treatments throughout the growing season, to compare hoverfly preference of insectary plants over time. Number of hoverflies fluctuated throughout the season and insectary plant preferences changed when available (flowering) insectary plant species changed. On 26 Jul 2017, the first sample date, there were significantly more hoverflies observed on buckwheat 1 than on alyssum (Table 6). For the next two sample dates the data were not statistically significant at $\alpha = 0.05$, but cilantro had higher mean hoverfly density than alyssum. By 17 Aug 2017 the number of hoverflies observed on buckwheat was not significantly different from that observed on alyssum. Hoverfly density was low in all treatments on 5 Sep 2017. In contrast, on 19 Sept 2017 alyssum had the greatest hoverfly density of all the flowering treatments on any given sampling date which coincided with buckwheat no longer blooming. On the same date (19 Sep 2017) alyssum had significantly greater hoverfly density than cilantro 1, dill 1, and phacelia. However, a week later,

on 27 Sep 2017 hoverfly density on cilantro 1 was significantly greater than alyssum, dill 1 and phacelia. Hoverfly density remained low on phacelia throughout the season. By mid-October hoverfly populations were dwindling on all treatments.

Weather data 2017-2018

Since it is possible that weather may affect the number of insects observed throughout the season, weather data from Network for Environment and Weather Applications (NEWA) online database were compiled from 10:00 AM to 12:00 PM during sample days in 2017 and 2018 (Table 7 and Table 9). Weather data were used to cross reference the patterns of number of hoverflies found per date. On 5 Sep 2017 mean hoverfly densities were low in all treatments and this observation date appeared to be an outlier (Table 7) We first hypothesized that we could attribute the low hoverfly density on 5 Sep 2017 to weather patterns, however, no apparent patterns were found. The weather data also confirmed that average windspeeds were equal or less than 5 miles an hour except for the very last sample date on 25 Oct 2018 (Table 9).

Table 6. Mean hoverfly density per 314 in^2 per four-minute interval in Durham, NH in 2017. Only actively flowering treatments were observed for number of hoverflies. Comparisons were made within date. Cilantro 2 and Dill 2 were excluded from this table because they began to bloom after the last observation date. Fennel was excluded because it did not flower.

Treatment	**26 Jul 17**	**2 Aug 17**	**10 Aug 17**	**17 Aug 17**	**5 Sep 17**	**19 Sep 17**	**27 Sep 17**	**23 Oct 17**
Alyssum	1.3 b[z]	3.7	10.0	5.0 ab	0.0	24.3 a	5.5 b	1.8
Buckwheat 1	15.5a	18.3	17.5	7.5 a	-	-	-	-
Buckwheat 2	-[y]	-	-	10.8 a	1.3	-	-	-
Cilantro 1	-	-	-	0.0 b	0.0	8.3 b	16.3 a	2.3
Dill 1	-	-	-	-	1.8	7.0 b	5.5 b	0.0
Phacelia	-	-	-	-	0.0	1.0 c	0.8 b	0.8
mean by date	8.4	11.0	13.8	5.8	0.6	10.1	7.0	1.2
sum by date	16.8	22.0	27.5	23.3	3.0	40.5	28.0	4.8
p	**0.0409**	**0.0725**	**0.4549**	**0.0033**	**0.1763**	**<0.0001**	**0.0080**	**0.2523**
F	11.9	7.4	0.7	9.9	1.9	60.0	7.5	1.6
df	1,3	1,3	1,3	3,9	4,12	3,9	3,9	3,9
SEM	3.1	3.1	4.8	1.9	0.6	1.2	2.5	0.9

[z] Within column, treatment means followed by the same letter are not significantly different according to Tukey's HSD or the Student's T-test (when comparing only two means) at $\alpha = 0.05$.

[y] Treatments with dashes were not observed on given dates because treatments were not actively flowering.

Table 7. Mean of abiotic factors from 10:00 AM to 12:00 PM during sample days in Durham, NH in 2017. Information sourced from Network for Environment and Weather Applications (NEWA).

Date	26 Jul 17	2 Aug 17	10 Aug 17	17 Aug 17	5 Sep 17	19 Sep 17	27 Sep 17	23 Oct 17
Air Temperature[z]	76	81	78	72	75	64	79	55
Leaf Wetness[y]	0	0	0	0	0	25	39	0
Relative Humidity[x]	51	60	63	51	74	97	76	87
Wind Speed (mph)	2	3	2	5	3	3	2	2
Wind Direction[w]	83	109	195	291	210	42	192	185
Solar Radiation[v]	66	61	57	26	43	6	39	9
Dewpoint[u]	56	66	64	52	66	63	71	51

[z] Air temperature – measured in Fahrenheit, the ambient air temperature

[y] Leaf wetness – the number of minutes that presence of free water was on the surface of a plant. This could be due to rainfall, fog, or dew.

[x] Relative humidity – the amount of water vapor present in air expressed as a percentage of the amount needed for saturation at the same temperature.

[w] Wind direction— measured in azimuth degrees (0° to 360°), wind is measured in degrees clockwise from due north. Wind blowing from the north has a wind direction of 0°, wind blowing from the east has a wind direction of 90°, wind blowing from the south has a wind direction of 180° and wind blowing from the west has a direction of 270°.

[v] Solar radiation – measured in Langleys (Ly), solar radiation is a unit of heat transmission received by the earth.

[u] Dewpoint – measured in Fahrenheit, the atmospheric temperature below which water droplets begin to condense, and dew can form.

Effect of insectary plants on hoverfly density over time: comparison by date, 2018

The total number of hoverfly observations on insectary plants fluctuated throughout the season and peaked during the month of August. There were three more sample dates than the previous year and significant differences between treatments were present on all sample dates except for 9 Aug 2018.

Alyssum had significantly greater hoverfly density or was not significantly different from other treatments on 9 out of 11 dates, buckwheat on 4 out of 6 dates, cilantro on 5 out of 10 dates, dill on 3 out of 8 dates, and calendula on zero out of 11 dates (Table 8 and Figure 19)

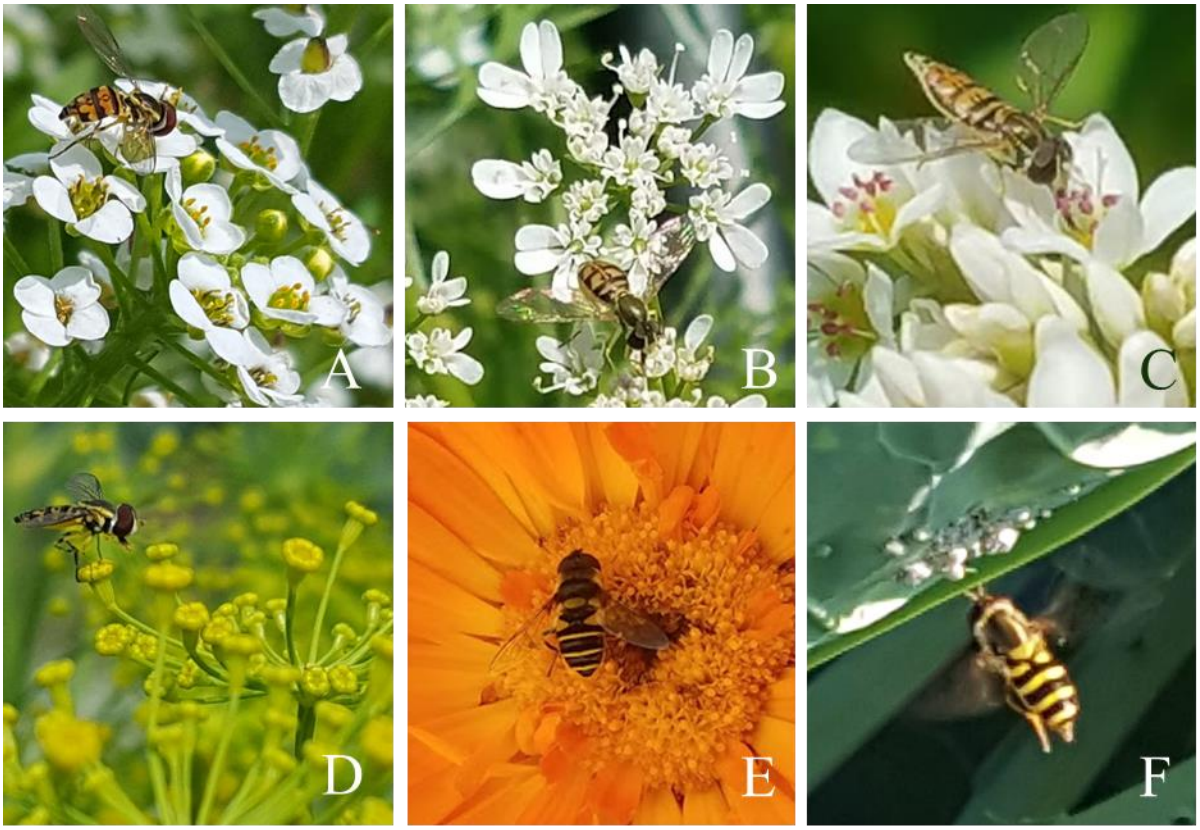


Figure 19. Hoverflies foraging on various insectary plants and locating cabbage aphids. (A) Toxomerus geminatus on alyssum; (B) Toxomerus marginatus on cilantro; (C) Toxomerus marginatus on buckwheat; (D) Toxomerus marginatus on dill; (E) Unidentified hoverfly on calendula; (F) Unidentified hoverfly locating an aphid colony on a Brussels sprout leaf.

On 27 Jul 2018, the first sample date, alyssum had a significantly greater hoverfly density than calendula 1 and dill 1. On the following date, 9 Aug 2018, all plant species were in flower and alyssum had the greatest hoverfly density, but differences were not statistically significant at $\alpha = 0.05$ (Table 8).

The mean hoverfly density on all treatments combined by date were greatest on 16 Aug 2018, 20 Aug 2018, and 27 Aug 2018. On 16 Aug 2018 cilantro 1 had significantly more hoverfly density (34.3 hoverflies/314 in^2/4 min) than alyssum (14.5), buckwheat 2 (11.3), and calendula (2.0), whereas the number of hoverflies observed on dill 1 and buckwheat 1 (18.3 and 17.8) were intermediate. On 20 Aug 2018 cilantro1 had significantly greater hoverfly density than calendula (7 times more), whereas, dill 1, alyssum, buckwheat 1 and buckwheat 2 were observed to have intermediate hoverfly densities (Table 8).

Alyssum was maintained hoverfly densities that were greater than all other treatments for the remaining 5 dates in 2018. On 27 Aug 2018 alyssum (17 hoverflies/314 in^2/4 min) had a significantly greater hoverfly density than calendula 1 (7.5) whereas there were no significant differences between alyssum, dill 1, cilantro 1, buckwheat 1 and buckwheat 2 (Table 8). The only sampling date that yielded significant differences while all species were in bloom was 13 Sep 2018. On that date, alyssum (16.8) had more than double to five times the hoverfly density compared to buckwheat 3, cilantro 1, calendula 1, buckwheat 1, buckwheat 2 and dill 2 (Table 8). For the remainder of the year alyssum had significantly greater hoverfly density than all treatments except for cilantro which was not significantly different than alyssum on two of the remaining dates (Table 8).

Insectary plants significant differences of 2017-18 combined

Including all 2017 and 2018 dates, the hoverfly density on alyssum was greater than or not significantly different from the treatment with the highest density on 11 of out 19 dates (Table 6 and Table 8). The hoverfly density on buckwheat was greater than or not significantly different from other treatments on 6 out of 11 dates, cilantro on 6 out of 15 dates, and dill on 3 out of 12 dates (Table 6 and Table 8). The denominator of these ratios differs by plant species because plant species have different length of bloom periods. Phacelia was grown only in 2017 and calendula was grown only in 2018. During these single years, hoverfly density on phacelia and calendula were not greater than other insectary plants on a single date, despite their long bloom periods. Fennel did not flower in 2017 and could not be evaluated.

Cumulative mean hoverfly density over entire season

In 2017, cumulative hoverfly density divided by weeks in bloom showed that alyssum and buckwheat had a significantly greater hoverfly density than phacelia, cilantro and dill (Figure 20). In 2018, alyssum again had significantly greater hoverfly density than all other treatments (Figure 20). Calendula experienced only 20% of the hoverfly density that alyssum experienced whereas cilantro, buckwheat, and dill had intermediate hoverfly density (Figure 20).

Cumulative mean hoverfly density over the entire season per treatment.

In the present study, alyssum and buckwheat had a significantly greater hoverfly density than other insectary plants in 2017 whereas in 2018 alyssum had significantly greater hoverfly density than all other insectary plant treatments and buckwheat was an intermediate treatment (Figure 20).

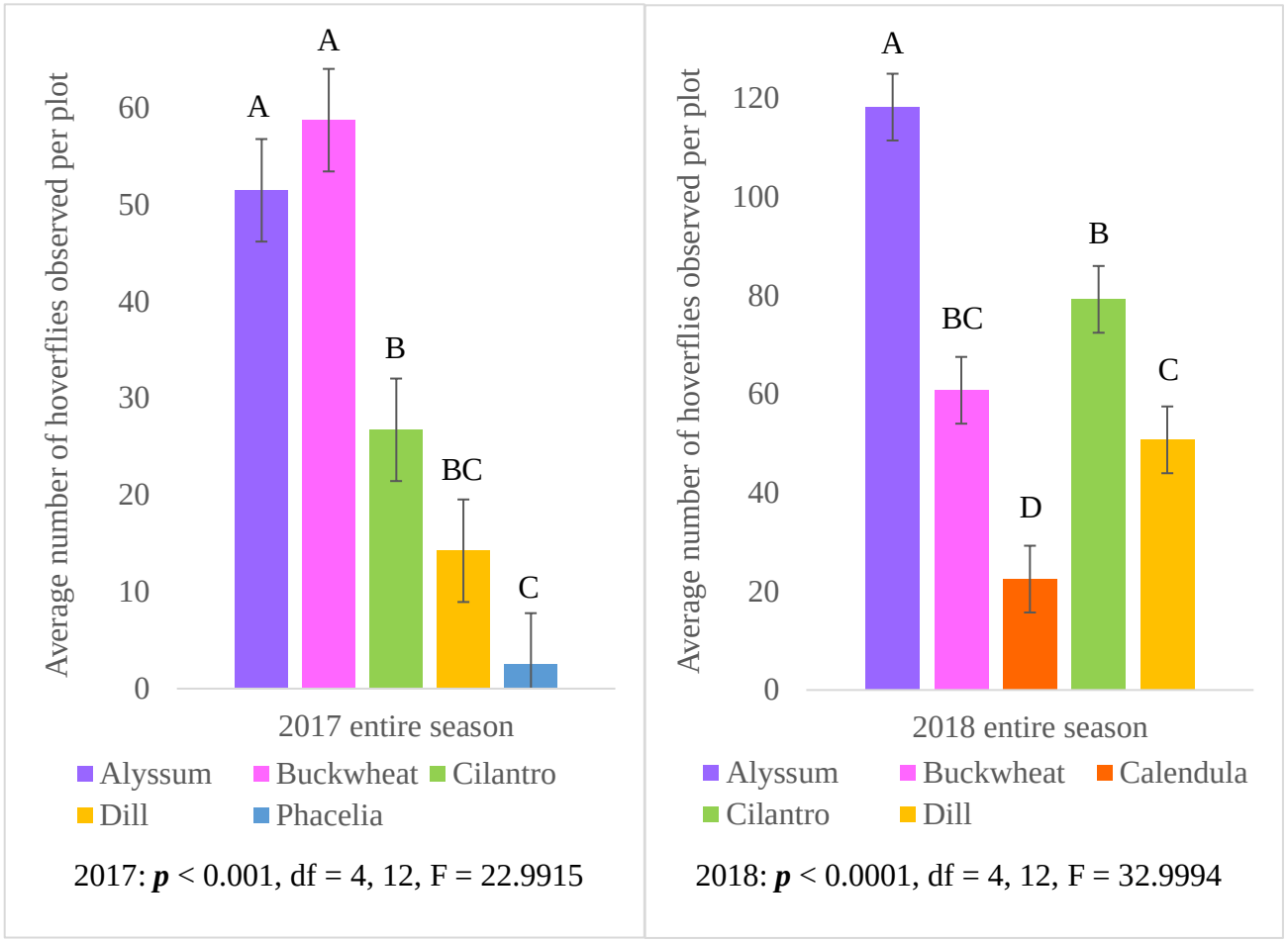


Figure 20. Cumulative mean hoverfly density over entire season per plot, 2017 and 2018. Treatment means followed by the same letter are not significantly different according to Tukey's HSD at α =0.05. Note: the y-axis is on different scales for each year.

Table 8. Mean hoverfly density per 314 in^2 per four-minute time interval in Durham, NH in 2018. Only actively flowering treatments were observed. Comparisons were made solely by date.

Treatment	27 Jul	9 Aug	16 Aug	20 Aug	27 Aug	13 Sep	20 Sep	27 Sep	4 Oct	10 Oct	19 Oct
Alyssum	10.3a[z]	13.0	14.5b	11.8ab	17.0a	16.8a	2.8a	7.8a	6.3a	13.5a	4.8a
Buckwheat 1	6.0ab	11.8	17.8ab	10.0ab	9.3ab	4.4b	-	-	-	-	-
Buckwheat 2	-[y]	6.5	11.3b	8.0ab	9.8ab	2.9b	-	-	-	-	-
Buckwheat 3	-	-	-	-	7.0b	6.5b	0.5b	-	-	-	-
Calendula 1	0.0b	3.0	2.0b	2.8b	7.5b	5.5b	0.0b	0.3b	0.3b	1.3c	0.3b
Calendula 2	-	-	-	-	-	-	-	-	-	1.3c	0.0b
Cilantro 1	-	3.5	34.3a	18.5a	15.3ab	6.3b	1.0ab	1.9ab	-	-	-
Cilantro 2	-	-	-	-	-	-	-	0.0b	0.5b	7.0b	0.5b
Dill 1	1.6b	4.5	18.3ab	12.0ab	13.0ab	2.8b	-	-	-	-	-
Dill 2	-	-	-	-	-	-	-	-	0.3b	1.0c	-
mean by date	4.5	7.0	16.3	10.5	11.3	6.4	1.1	2.5	1.8	4.8	1.4
sum by date	17.8	42.3	98.0	63.0	78.8	45.1	4.3	9.9	7.3	24.1	5.5
p	**0.003**	**0.074**	**0.003**	**0.016**	**0.013**	**<0.0001**	**0.006**	**0.004**	**0.0002**	**<0.0001**	**0.0002**
F	10.5	2.5	6.3	4.0262	3.77	10.61	6.24	8.59	13.61	43.28	22.27
df	3, 9	5,15	5,15	5,15	6,18	6, 16.99	4, 12	4, 8.82	4,12	4, 11.28	3,9
SEM	1.65	2.98	4.84	2.49	2.10	2.05	0.48	1.14	0.72	0.83	0.42

[z]Within column, treatment means followed by the same letter are not significantly different according to Tukey's HSD or the Student's T-test (when comparing only two means) at $\alpha = 0.05$.

[y]Treatments with dashes were not observed on given dates because treatments were not actively flowering.

Table 9. Mean of abiotic factors from 10:00 AM to 12:00 PM during sample days in Durham, NH in 2018. Information sourced from Network for Environment and Weather Applications (NEWA).

Date	27 Jul	9 Aug	16 Aug	20 Aug	27 Aug	13 Sep	20 Sep	27 Sep	4 Oct	10 Oct	19 Oct	25 Oct
Air Temperature[z]	81	79	80	72	80	72	60	65	63	76	45	41
Leaf Wetness[y]	0	3	0	0	0	0	0	0	0	0	0	0
Relative Humidity[x]	78	81	70	67	67	77	87	68	92	76	66	66
Wind Speed (mph)	2	5	5	4	5	1	3	3	3	5	4	10
Wind Direction[w]	188	271	293	81	311	190	65	48	181	270	245	303
Solar Radiation[v]	64	53	48	59	56	35	23	49	23	40	39	29
Dewpoint[u]	73	72	70	61	68	64	56	54	61	68	34	31

[z] measured in Fahrenheit, the ambient air temperature

[y] Leaf wetness – the number of minutes that presence of free water was on the surface of a plant. This could be due to rainfall, fog, or dew.

[x] Relative humidity – the amount of water vapor present in air expressed as a percentage of the amount needed for saturation at the same temperature.

[w] Wind direction— measured in azimuth degrees (0° to 360°), wind is measured in degrees clockwise from due north. Wind blowing from the north has a wind direction of 0°, wind blowing from the east has a wind direction of 90°, wind blowing from the south has a wind direction of 180° and wind blowing from the west has a direction of 270°.

[v] Solar radiation – measured in langleys (Ly), solar radiation is a unit of heat transmission received by the earth.

[u] Dewpoint – measured in Fahrenheit, the atmospheric temperature below which water droplets begin to condense, and dew can form.

Effect of insectary plants on cumulative density of other insects, 2017

There were significant differences between cumulative insect density per treatment for bees, honeybees, bumble bees, other large hymenoptera, lady beetles, ants, other flies, fleabeetles, and spiders (Figure 27).

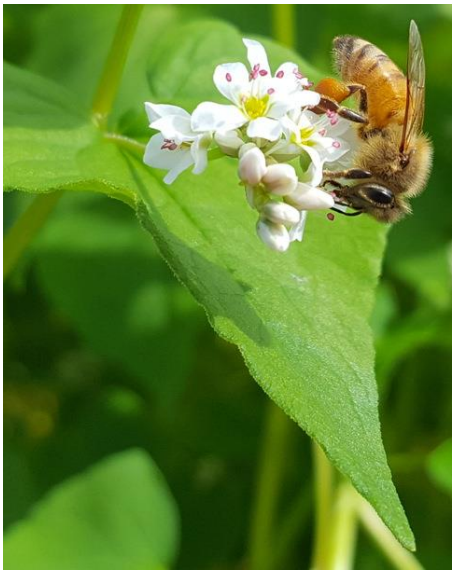


Figure 21. A honeybee feeding from buckwheat flowers.

Honeybees. Buckwheat had a significantly greater honeybee density than all other treatments ($p<0.0001$). There were zero honeybees observed on dill, while alyssum, cilantro, and phacelia had intermediate honeybee densities (Figure 21 and Figure 27).

Bumble bees. Phacelia had a significantly greater bumble bee density than all treatments except for cilantro which was not significantly different than phacelia ($p=0.0008$) (Figure 22A and Figure 27). There were zero bumble bees observed on alyssum, whereas buckwheat and cilantro had intermediate bumble bee densities (Figure 27).

Figure 22. Non-target insects feeding on phacelia flowers. (A) bumble bee; (B) other bee.

Other bees. Buckwheat had a significantly greater hoverfly density than any other treatment (p<0.0001). Phacelia had an intermediate hoverfly density, whereas there were zero bees observed on cilantro and dill (Figure 22B and Figure 27).

Other large hymenoptera. Other large hymenoptera were observed on all treatments. Dill was had a significantly greater hymenoptera density than alyssum (p=0.0421) (Figure 23 and Figure 27), while the number of hymenoptera observed on cilantro, buckwheat and phacelia were intermediate and not significantly different from dill or alyssum (Figure 23 and Figure 27).

Figure 23. Other large hymenoptera on various insectary plants. (A) dill; (B) alyssum; (C) cilantro; (D) buckwheat.

Lady beetles. Lady beetle larvae and adults were observed on all treatments except cilantro, which had zero observations of lady beetles. Dill had a significantly greater ladybeetle density than all other treatments.(p=0.0019) (Figure 24 and Figure 28).

Figure 24. Lady beetle adults on dill plant and lady beetle adult foraging on dill flower.

Ants. Ants were observed on all treatments. The number of ants observed on buckwheat was significantly greater than the number of ants observed on cilantro, dill, and phacelia (p=0.0067). There was an intermediate number of ants observed on alyssum (Figure 28).

Fleabeetles. There were significantly more fleabeetles observed on alyssum than all other treatments (p=0.0034) (Figure 28). Alyssum showed obvious signs of infestation during the beginning of the season (Figure 25).

Figure 25. Obvious fleabeetle infestation and damage on the flowers of alyssum; petals have been eaten.

Spiders. There were significantly more spiders observed on alyssum than all other treatments, except for cilantro which had equal or fewer observations of spiders than alyssum (p=0.0171) (Figure 28).

Other flies. Other flies were observed on all treatments. Alyssum had a significantly greater fly density than all other treatments (p=0.0016) (Figure 26 and Figure 28).

Figure 26. Other flies on alyssum flowers (A) Tachinid fly (Diptera: Tachinidae); (B) unknown fly (Diptera).

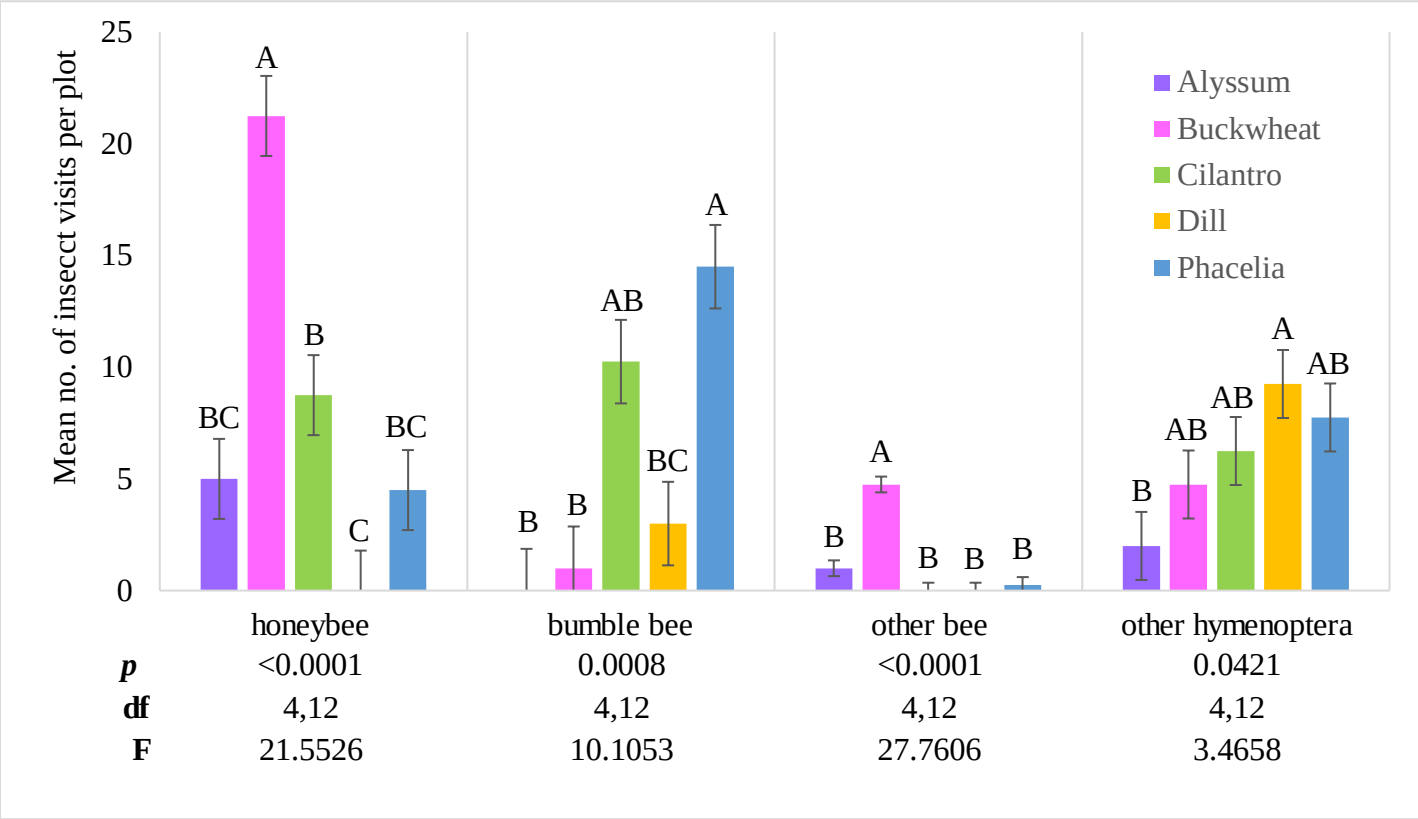


Figure 27. Mean honeybee, bumble bee, other bee and other large hymenoptera visitation over entire season per plot in 2017. For each insect grouping, treatment means noted with the same letter are not significantly different according to Tukey's HSD at α =0.05. Means comparisons are only made within insect groupings. For treatments with succession plantings, only the first succession was used.

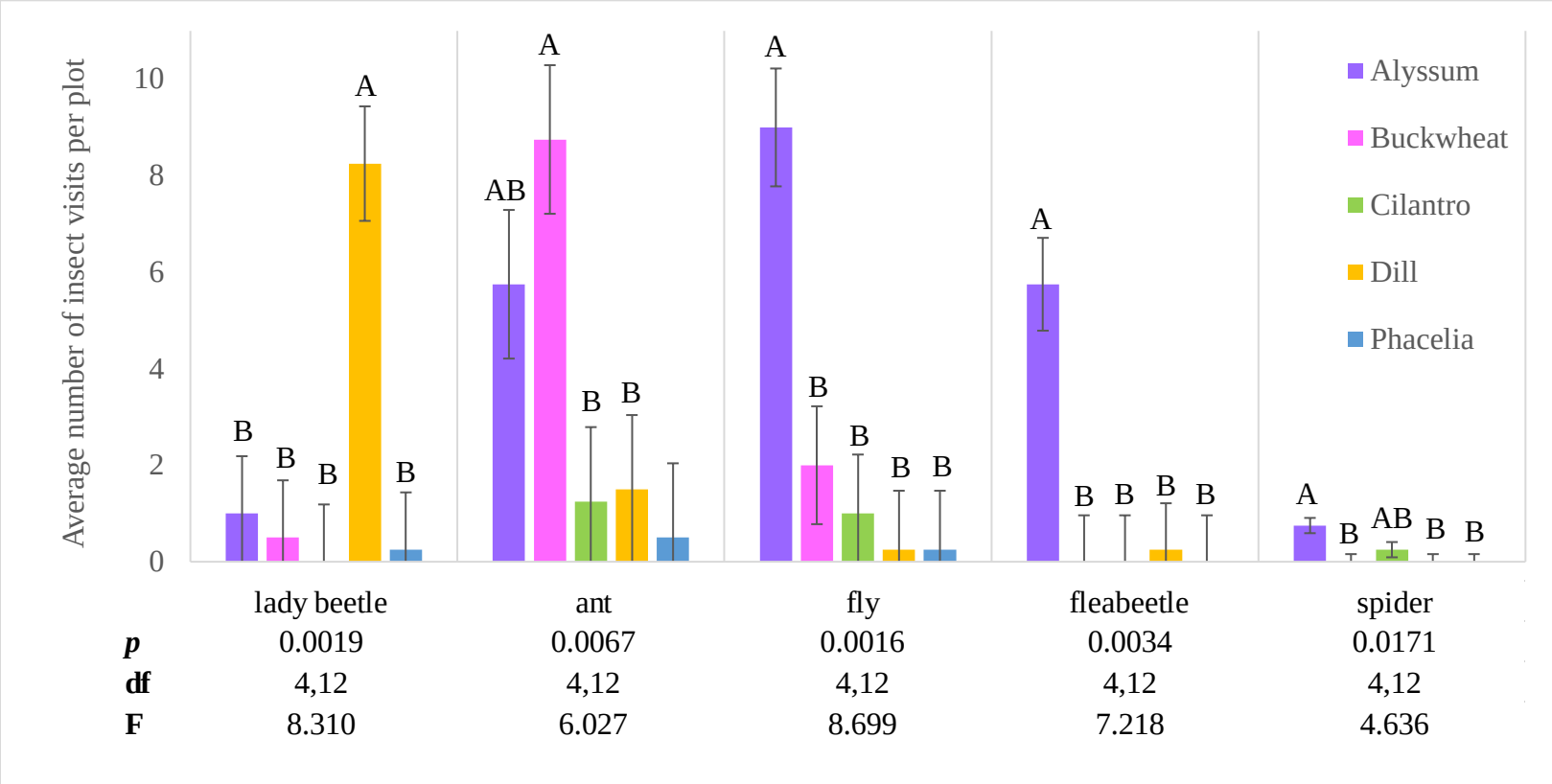


Figure 28. Mean lady beetle, ant, fleabeetle, and spider visitation over entire season per plot in 2017. For each insect grouping, treatment means noted with the same letter are not significantly different according to Tukey's HSD at α =0.05. Means comparisons are only made within insect grouping. For treatments with succession plantings, only the first succession was used.

Effect of insectary plants on cumulative other insect density, 2018

There were significant differences between cumulative mean insect density over the entire season for honeybees, bees, bumble bees, and imported cabbageworm moth (Figure 30).

Honeybees. Buckwheat and cilantro had a significantly greater honeybee density than dill while alyssum and calendula had an intermediate number of honeybees observed (p=0.0192). Dill had a lower number of honeybees observed with a cumulative mean density of less than one hoverfly per plot. The number of honeybees observed on buckwheat and cilantro were significantly greater than dill (p=0.0192)(Figure 30).

Bumble bees. Calendula had significantly greater bumble bee density than all insectary plant species (p=0.0017). There were zero bumble bees observed on alyssum and dill. Honeybee densities on buckwheat and cilantro were intermediate (Figure 30).

Other bees. Other bees were observed on all treatments. Calendula had a significantly greater other bee density than alyssum and dill, while buckwheat and cilantro had intermediate other bee densities (p=0.0233). Calendula had four times the bee density as dill (Figure 30).

Lady beetles. Lady beetle adults and larvae were observed on all treatments except for calendula where zero ladybeetles were observed. However, there were no significant differences between treatments (p=0.0017) (Figure 30).

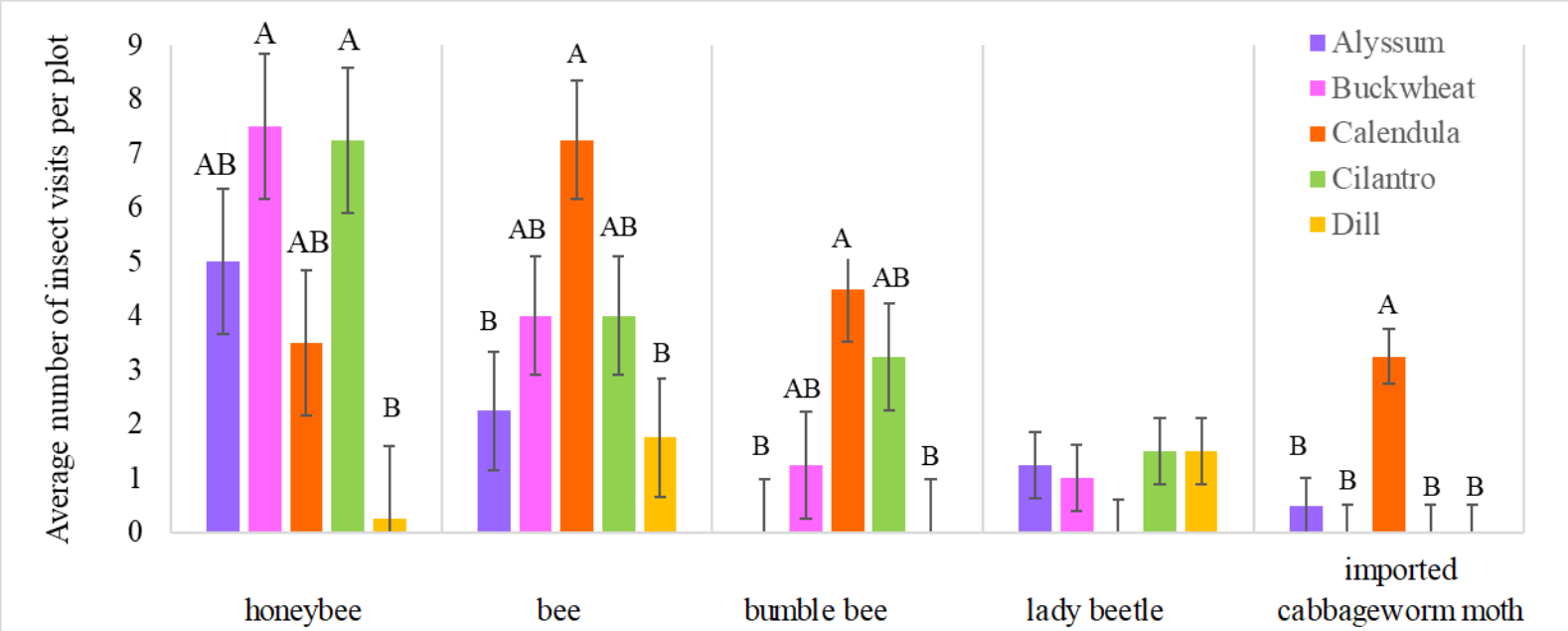
Imported cabbageworm moth (Pieris rapae). Calendula had the greatest number of observed imported cabbageworm moth, a lepidopteran pest of brassica crops (Figure 29 and Figure 30). There were zero imported cabbageworm moths observed on cilantro and dill throughout the year, whereas alyssum and buckwheat had an intermediate density of imported cabbageworm but significantly less than calendula (p=0.0022) (Figure 30).

Insectary plant selective attraction to insects. The number of insects observed on dill and calendula showed selectivity in the groupings of insects cumulatively throughout the year.

Though dill had only moderate hoverfly densities, it had fewer honeybee, bumble bee ad other bee density compared to other insectary plants with high hoverfly densities (buckwheat, alyssum, cilantro). Furthermore, lady beetle adults were observed on dill in both years and in 2017 dill had a significantly greater lady beetle adult and larvae density than all other plant species (p=0.0019) (Figure 28).

The hoverfly density of calendula was significantly lower than all other treatment whereas calendula had significantly greater imported cabbageworm moth, (Figure 29), bumble bee and other bee densities than alyssum and dill.

Figure 29. Imported cabbageworm moths feeding on calendula insectary plants. The caterpillar stage of this moth eats Brassica crops, such as Brussels sprouts.



	honeybee	bee	bumble bee	lady beetle	imported cabbageworm moth
***P* value**	0.0192	0.0233	0.0091	0.3807	0.0022
df	4, 12	4, 12	4,12	4,12	4,12
F	4.47	4.21	5.56	1.15	8.00

Figure 30. Mean honeybee, bee, bumble bee, lady beetle, and imported cabbageworm visitation over entire season per plot in 2018. For each insect grouping, treatment means notated with the same letter are not significantly different according to Tukey's HSD at α =0.05. Means comparisons are only made within insect grouping. For treatments with succession plantings, only the first succession was used.

Insectary plant seedling production

We observed that seeding 2 buckwheat seeds per cell resulted in thin stands with lighter flower density in the field than desired. Additionally, the 128 cell trays were very prone to drying out. Sub-irrigation may be helpful for future experimentation. Direct seeding with higher seed density achieved adequate stands of flowers.

Hoverfly adult identification

Based on sweepnet samples of 222 captured and identified hoverflies specimens over 10 dates, the most abundant hoverfly found throughout the 2018 season was *Toxomerus marginatus* which represented 84.2% of all hoverfly specimen collected through the sweepnet. *Syritta pipiens* and *Toxomerus politus* each represented 3.6% of all hoverfly specimen collected. The remaining 5% of hoverflies was comprised of *Toxomerus geminatus*, *Syrphus ribesii*, *Syrphus vitripennis*, *Sphaerophoria philanthus*, *Sphaerophoria contigua*, *Eupeodes americanus*, *Melanstoma mellinum*, *Eristalis transversa* and *Eristalis tenax* (Figure 31 and Table 10). There was another hoverfly species from the Sphaerophoria genus could not be identified with only male specimens but was not prevalent in our sweepnet samples.

Cabbage aphids

In 2017 there were substantial populations of cabbage aphids on the Brussels sprouts, whereas, in 2018 there were very few cabbage aphids.

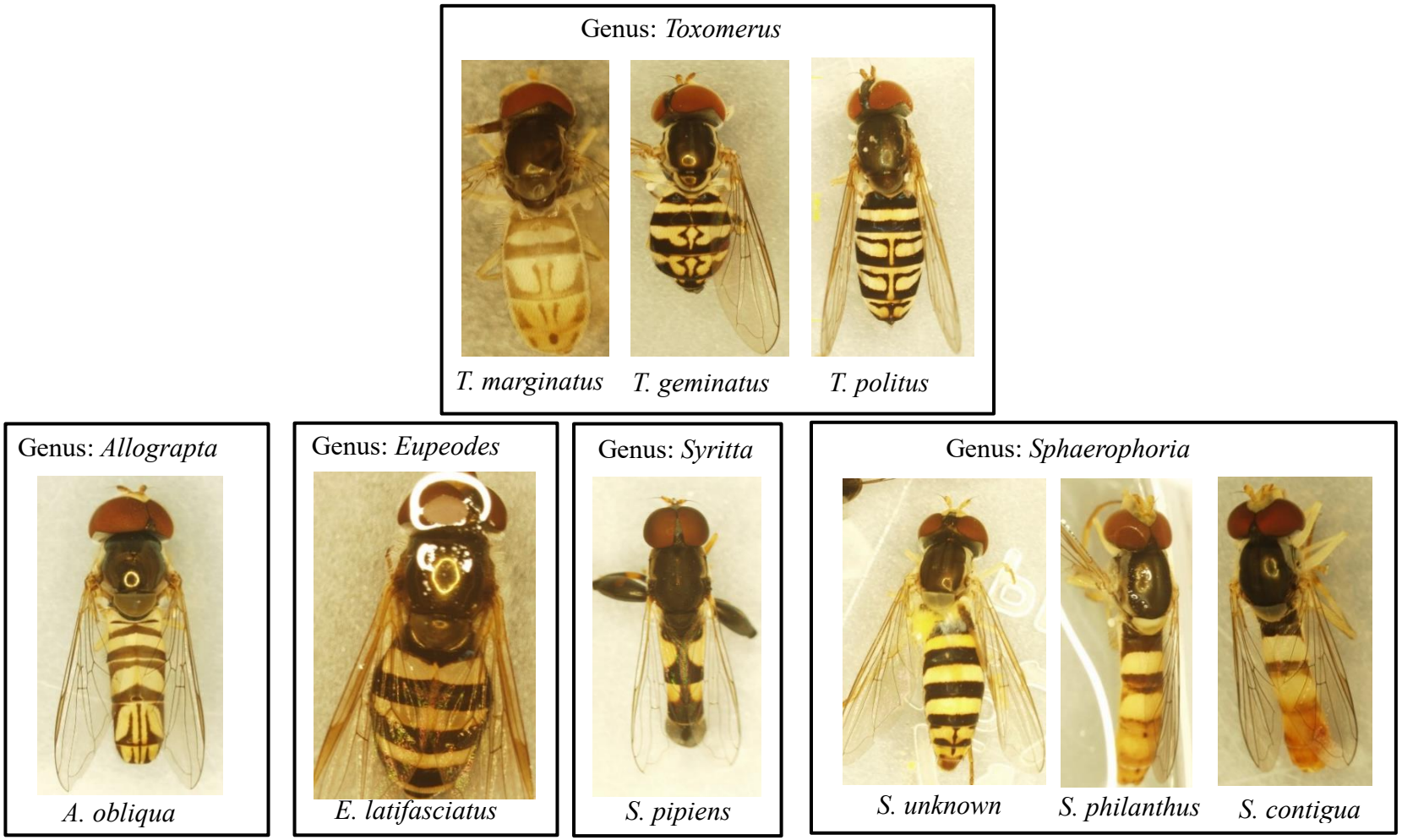


Figure 31. Microscope photos of hoverfly adult specimen by Genevieve Higgins and Sue Scheufele. We collaborated with the University of Massachusetts using the same sweepnet protocol. All hoverflies pictured were found in both Massachusetts and New Hampshire except for E. latifasciatus. A. obliqua are not included in the 2018 sweepnet table but were found in 2017 and 2019. One hoverfly species from the Sphaerophoria genus could not be identified using only male specimens.

Table 10. Number of hoverfly species collected per eight sweepnet samples per insectary plant treatment over time, 2018.

Treatment	Date	*Toxomerus marginatus*	*T. geminatus*	*T. politus*	*Syritta pipiens*	*Syrphus ribesii*	*Syrphus vitripennis*	*Sphaerophoria contigua*	*Sphaerophoria philanthus*	*Sphaerophoria unknown*	*Eupeodes americanus*	*Melanostoma mellinum*	*Eristalis transversa*	*Eristalis tenax*	Total by date
Alyssum	30 Jul 18	4													4
Alyssum	20 Aug 18	19													19
Alyssum	27 Aug 18	8													8
Alyssum	23 Sep 18	4			1					1					6
Alyssum	27 Sep 18	4													4
Alyssum	30 Sep 18	11					1				1				13
Alyssum	5 Oct 18	12							1						13
Alyssum	6 Oct 18	1			3										4
Alyssum	9 Oct 18	25			1	1	1								28
Alyssum	19 Oct 18	23													23
Buckwheat I	9 Aug 18	6													6
Buckwheat I	20 Aug 18	6			1			1							8
Buckwheat II	20 Aug 18	4		1											5
Buckwheat I	27 Aug 18	1													1
Buckwheat II	27 Aug 18	1		1											2
Buckwheat III	27 Aug 18	1													1
Calendula I	30 Jul 18	1													1
Calendula I	20 Aug 18	1													1
Calendula I	27 Aug 18												1		1
Calendula II	23 Sep 18	1													1
Calendula II	30 Sep 18	1													1
Calendula I	5 Oct 18	1													1
Calendula II	5 Oct 18	1												1	2
Calendula II	6 Oct 18	1				1	1								3
Calendula I	9 Oct 18	1												1	2
Calendula II	9 Oct 18	2					1		1	1					5
Cilantro I	20 Aug 18	8		2											10
Cilantro I	27 Aug 18	8		1	1										10
Cilantro II	30 Sep 18	3													3
Cilantro II	5 Oct 18		1												1
Cilantro II	6 Oct 18	3													3
Cilantro II	9 Oct 18	1			1						1	1			4
Cilantro II	19 Oct 18										1				1
Dill I	30 Jul 18	4													4
Dill I	20 Aug 18	11		1											12
Dill I	27 Aug 18	9		2											11
Total number by species		187	1	8	8	2	4	1	2	2	3	1	1	2	222
Percent collected in 2018		84.2%	0.5%	3.6%	3.6%	0.9%	1.8%	0.5%	0.9%	0.9%	1.4%	0.5%	0.5%	0.9%	

DISCUSSION

Continuous Bloom Period. Succession plantings of several plant species resulted in at least one species continuously blooming, however, there are material and labor drawbacks. Each plant species must be purchased, sown and given organizational attention and labor to achieve the planting schedules used in this experiment. A more practical compromise between labor and material costs as well as a continuous bloom period is using a single planting of alyssum. Alyssum's physiology is indeterminate; it continues to flower all season long, despite setting seed. It flowered for the entire period in which we wanted to enhance hoverfly numbers in the field and was consistently had moderate to high hoverfly densities. Hogg et al. (2011a) also found alyssum and phacelia to have the longest bloom period of eight other plants evaluated (buckwheat, wild mustard (*Brassica* sp.), wild arugula (*Diplotaxis muralis*), Thai basil (*Ocimum basilicum* var. *thyrsiflora*), white borage (*Borago officialis* 'Alba'), cosmos (*Cosmos sulphureus*) and nasturtium (*Tropaelum majus* 'Milkmaid')).

Furthermore, alyssum had the greatest hoverfly density in 11 out of 19 dates (Table 6 and Table 8). compared to other treatments. This implies that using solely one planting of alyssum in the beginning of the season as a continuous floral resource may be adequate to enhance hoverfly numbers in crop fields without the added labor and seed costs of planting other species. Cowgill et al. (1993) also discuss the potential use of seed cocktails with multiple insectary place species that allow for a single planting date with a continuous and diverse supply of food sources. We propose the potential of an alternative approach of seed cocktails that mix accessions and/or cultivars of a single species to lengthen bloom through differing days to maturity (i.e. cilantro).

Floral resources earlier in the season. Cowgill et al. (1993) suggest that it might be important to provide floral resources earlier in the year to enhance earlier oviposition from the overwintering generation of hoverflies in the spring. Colley and Luna (2000) also state that the ideal insectary plant should blossom in the beginning of the crop cycle to attract and establish natural enemies before infestations. In 2016, 2017, and 2018 the first winged aphids migrated into the field in early to mid-July, which roughly coincided with the beginning of insectary plant bloom period. It is possible that earlier establishment of insectary plants is important to support biological control agents of cabbage aphid. For example, hoverflies must first feed from flowers before they (1) mate, (2) lay eggs, and (3) their young hatch. Only after these steps can a hoverfly be an effective biological control agent, which underlines the importance of early bloom establishment.

Bolt-resistant cultivars of insectary plants. The only cilantro readily available to us was "bolt" or "heat" resistant, meaning that it has been bred to resist flowering during the hot days of summer. This resulted in cilantro beginning to bloom only in mid-August. It would be ideal to use a cultivar bred for the quick formation of coriander seed. Since this type of cultivar is not likely to be readily available, we conclude that 'Santo' cilantro must be seeded early in the season for it to function as an insectary plant when cabbage aphid arrives.

Similarly, the fennel cultivar used was also "bolt resistant" and did not flower during the growing season. It served as a lady beetle attractant and habitat despite never flowering. Although fennel encouraged beneficial predacious insects, one of the purposes of this insectary planting was to provide nectar and pollen. Potential solutions to this include use of a different cultivar, or an earlier planting date which may need to be a year prior, depending on cultivar or hardiness zone. Colley and Luna (2000) report successful flowering of fennel in Oregon, United

States between late July and August within the same year when sown on 25 March 1997, however, they may have used a different cultivar of fennel than in this study.

Considering the effect of methods on results

In this study, hoverflies did not need to land or feed on insectary plant species to be counted in hoverfly density data. In contrast, Colley and Luna (2000) state "Hoverflies entering the plot and feeding from flowers were counted as feeding visits," which we assume means only hoverflies feeding on flowers were counted, not just simply entering the plot. In this study, we counted "fly through" insects to reflect that insectary plants are not only as used as food resources, but also potentially as a preferred habitat, shelter, or mating site for insect predators or parasitoids (Altieri and Whitcomb, 1979; Wäckers and van Rijn, 2012). Wäckers and van Rijn (2012) also speculate that hoverflies could seek flowers for heat since reflection or concentration of infrared radiation from the sun and chemical reactions can produce heat in flowers (Wäckers and van Rijn, 2012). It is important to note that hoverfly ratios could be different in this study that counts "fly throughs" compared to other studies that count only the hoverflies that are feeding from flowers.

Experimental design can change the results or interpretation of preference experiments. Our randomized complete block experimental design is a "choice test" that seeks to compare the preference of multiple plant species to an organism (i.e. hoverfly) simultaneously (Murray, 2004). Since several plant species are evaluated at the same time, this measures attractiveness to hoverflies when presented with several choices; not whether they are attracted to the plant species without choices or with different choices. These experiments present a *preference*, as opposed to understanding if hoverflies will feed on a given species. For example, if hoverfly density is greater on alyssum compared with calendula, researchers must be careful to not

conclude that hoverflies do not utilize calendula, but rather that they prefer alyssum when both choices are available. If calendula was the only plant presented to hoverflies, it is possible that hoverfly density would increase despite it being a non-preferred plant species.

A way to narrow the results of a "choice test" design for future research is called "choice minus target." It omits the most preferred insectary plant and the experiment is repeated to find the next most preferred insectary plant. The experiment is repeated until all insectary plants are ranked. Another alternative variation of a "choice test" is a "sequential choice test" that would expose hoverflies to only one species at a time and repeat the experiment with a series of different species (Murray, 2004). Field studies inherently present variability because the surrounding environment cannot be controlled for abiotic conditions or other attractive plant or animal species.

Colley and Luna (2000) also used a randomized complete block design of flowering insectary plants with four replication showed selectivity among flowering species and this feeding preference changed when the flowering species available changed. They use cilantro and fennel as an example: when cilantro ceased flowering the feeding preferences shifted from cilantro to fennel. In this study, we also encountered similar shifts in 2018. Number of hoverflies observed on alyssum were greater than all other treatments on 27 Jul 2018 whereas when cilantro was fully in bloom on 16 Aug 2018 it had over double the number of hoverflies observed on alyssum. By 13 Sep 2018, feeding preferences shifted to alyssum again when cilantro flowers were not as abundant. If hoverflies have multiple flower types to choose from, it allows for preferential feeding. Additionally, the surrounding miles of habitat could encourage or discourage other natural nectar and pollen competitors to forage from the same insectary plants as the hoverflies.

Observations were also taken before noon because (Gilbert 1985) reported 10:00 AM to 12:00 PM to have the highest hoverfly activity in the United Kingdom. However, it is possible that plant flower phenology is different among species during different times of the day. Further research is needed to understand when new nectar and pollen resources become available in a recently opened flower. It cannot be assumed that all open flowers have released pollen or nectar resources. Perhaps pollen and nectar resources become available later in the day for some plant species, in which case, hoverfly densities could be different if observed at different times of day.

We evaluated relative hoverfly preference of insectary plants to *all* species of adult hoverflies summed together. Since all hoverfly species were counted in the timed field observations, and hoverfly species presence and their preferences may have changed with the season, it is plausible that different planting dates or plant species may have affected the hoverfly density or mere presence of a given hoverfly species. In future experiments it would be helpful to winnow down the prospective hoverfly adults to focus specifically on the species that are known to eat aphids in their larval stage. Since it is difficult to identify hoverfly adults "on the wing," while moving in the field, we suggest two things in conjunction to understand the relationship between hoverfly species and cabbage aphid. First, we suggest the use of sweep nets to evaluate relative preference of insectary plants and identify aphid-eating hoverfly adults. Sweep netting in the Brussels sprout field may be possible, but not likely to yield many adult hoverfly specimens. Next, we suggest rearing out the larvae found on nearby Brussels sprouts to identify species and compare against the species collected from the insectary plants.

There was a large plot of alyssum abutting the experimental site and this may have affected results by drawing hoverflies away from the randomized complete block design of

insectary plants. Furthermore, it is possible that there is an effect of flower density on hoverfly plant preference within treatments or between treatments of the randomized complete block design. In the present study there were statistical differences in alyssum flower densities counted throughout the course of the bloom period. However, during these same observation dates, there were not statistical differences between hoverfly densities on alyssum tested against itself. This infers that hoverfly preference of alyssum does not change when there are differences in flower densities. Other insectary plants were not tested for these differences and may yield different results. However, our results of alyssum flower densities are in alignment with Gilbert (1981), Ruppert and Molthan (1991) and Cowgill et al. (1993) who reported that availability of flowers is independent of flower selectivity. Cowgill et al. (1993) summarized their three findings by saying hoverflies "did not visit flower forms according to their abundance in the habitat."

Visual observations of hoverflies on flowers have been criticized by Haslett (1989) due to the different "handling times" of different flowers and they advocate for the analysis of the gut contents (pollen grains) of hoverflies in lieu of visual observation. Cowgill et al. (1993) discusses that since the production of pollen varies greatly among plant species, that abundance flowers is not necessarily an accurate measurement of the amount of pollen available. We agree that gut analysis of sweep net specimens could be a crucial piece of information which could provide a cross-reference for visual observation of hoverflies on flowers. However, this method was not pursued in the current study. We also acknowledge that if gut contents were only analyzed for pollen grains, that flower nectar as a hoverfly food resource may be overlooked.

Hoverflies and natural enemies: preference of insectary plants in the literature

Hogg et al. (2011) found alyssum to consistently attract the most hoverfly visitation compared to other harvestable flowering plant treatments. Colley and Luna (2000) cite alyssum

to be a significant provider of floral resources for hoverflies, but cilantro had the greatest visitation rates in their study. MacLeod (1992) found cilantro and buckwheat to be the most attractive species to hoverflies. These results were largely consistent with our findings of hoverfly densities and slight differences are likely explained by hoverfly selectiveness based on what floral resources are available (Colley and Luna, 2000) or different species of hoverflies present.

Dill has a yellow flower, which is the color that has been demonstrated to be most attractive to aphid-eating hoverflies (Hoback et al., 1999; Kevan and Baker, 1983; Laubertie, 2007). However, Haslett (1989) criticizes the use of color descriptions as seen by the human eye, as compared to the insect visual spectrum. Haslett (1989) argues we can avoid misinterpretation of color by using reflectance spectra through use of a reflectance spectrophotometer. Haslett (1989) evaluated hoverfly preferences and found different hoverfly species to be attracted to an array of reflectance spectra colors (yellow, blue and violet, white).

Furthermore, dill is in the family *Apiaceae* which have umbelliferous flowers known to attract hoverflies, specifically *Episyrphus balteatus* (Wäckers and van Rijn, 2012). In the present study, dill had an intermediate number of hoverflies observed. However, *E. balteatus* was not identified as one of the hoverfly species collected in sweepnet samples, which may explain the differences in our results.

Lixa et al. (2010) report higher populations of lady beetle species on dill when compared with cilantro and fennel, which they partially attribute to plant odor and the yellow flower color (Wäckers and van Rijn, 2012). Laurenz and Meyhöfer (2016) also found dill to be more attractive than the seven other plant species evaluated. They reported there were 4.7 times more lady beetles found on dill than the other treatments, which included phacelia and buckwheat. In

the present study, lady beetles were observed on dill in 2018 but there were no significant differences between treatments.

Colley and Luna (2000) found phacelia to be a significant provider of floral resources for hoverflies, whereas, in the present study phacelia had very low hoverfly density in 2017 and was omitted in 2018 due to poor germination. Rijn and Wäckers (2016) report phacelia to have significantly lower zoophagous hoverfly visitation compared to 11 other flower choices. In another study, phacelia was also found to have the lowest zoophagous hoverfly visitation (hoverfly species: *Sphaerophoria scripta, Melanostoma mellinum, Syrphus ribesii, Syrphus Vitripennis, E. balteatus, Sphaerophoria ruepellii*) compared with seven other flowering species, including dill and buckwheat (Laurenz and Meyhöfer, 2016). Calendula attracted few hoverflies in other studies (Colley and Luna, 2000; Laubertie, 2007) as well as in this study.

Flower nutrition to hoverflies in the literature. Laubertie et al. (2012) reported that provision of alyssum flower to *E. balteatus* hoverflies resulted in a small proportion of successfully laid eggs and they ranked the net reproductive rate of hoverflies fed diets of phacelia, buckwheat, and cilantro to be superior to those fed alyssum. Hogg et al. (2011a) reported that without access to alyssum as a food source, *Eupeodes fumipennis* eggs either did not develop or were resorbed back into the body of the adult hoverfly. However, since Laubertie at al. (2012) and Hogg et al. (2011a) used two hoverflies that were not identified in our region for this fitness determination, we cannot draw conclusions about all species of hoverflies. Even hoverflies in the same genus (i.e. *Toxomerus*) do not necessarily eat the same food (Jordaens et al., 2015). Among several other zoophagous species of hoverflies, *E. balteatus* has been categorized as a pollen feeder, or pollen specialist (Branquart and Hemptinne, 2000; Gilbert and

Owen, 1990) in contrast with other species that include a greater proportion of nectar in their diet.

Since *E. balteatus* is commonly used in the literature as a model organism and was not identified to be present in the Durham, NH agroecosystem, we would like to emphasize the need for a future experiment that evaluates the effects of reproduction on *T. marginatus* fed an alyssum flower diet. Furthermore, Laubertie et al. (2012) found phacelia to have the highest net reproductive rate compared to four other insectary plant treatments in the present study. However, the present study had very low numbers of hoverflies found on phacelia throughout the year. Branquart and Hemptinne (2000) demonstrate a correlation between tongue length of hoverfly species and depth of corolla of the flowers. Since the architecture of the phacelia flower may affect the number of hoverflies that visit, it would be helpful to know the tongue length of the most abundant hoverfly in this study (*T. marginatus*).

Non-target insects: preference of insectary plants in the literature

Just as insectary plants could be a potential food resource to hoverflies, they could also serve as a resource for pests of *Brassica* crops. In the present study, in 2018 calendula displayed selectivity in a deleterious way with a significantly greater number of imported cabbageworm moths observed than other treatments, zero observations of lady beetles, and numbers of other pollinators (honeybees, bumble bees, other bees) that could potentially be competition for hoverflies.

Two field studies have evaluated competition for food resources among two species of bumble bees (*Bombus appositus* and *B. flavifrons*) (Inouye, 1978) and among bumble bees and honeybees (Balfour et al., 2015). Both studies found insects to display different foraging behavior when insect competitors were present and were found to forage more and on different

flowers when competitors were excluded. Balfour et al. (2015) describes this behavior as exploitative competition. Hogg et al. (2011) also concludes that competition with bees may have negatively affected hoverfly visitation rates to several of the insectary plants evaluated. Baggen et al. (1999) discusses "selective plants" that attract wanted insect species, but not competitors of food resources or pests. In the present study, dill had an intermediate hoverfly density and the densities of honeybees, bees, and bumble bees were low. We consider dill to be a beneficial selective plant in our experiment. Similarly, Hogg et al. (2011) reports alyssum to attract the highest visitation rates of hoverflies, while attracting the least number of bees (potential competitors). However, alyssum is in the *Brassica* family and may share pests with Brussels sprouts, the cash crop being studied for pest management. In 2017 we observed significantly more fleabeetles on alyssum compared with all other treatments. It is possible that fleabeetles could not only affect alyssum's performance as an insectary plant, but also, alyssum could serve as an attractant or host to the fleabeetles, which are a pest in *Brassica* crops. Baggen et al. (1999) states that an ideal insectary plant should attract fewer pests than the crop itself. There were fleabeetle infestations on Brussels sprout plants early in the year in 2017 which were counted on the Brussels sprouts leaves (Figure 32). However, since fleabeetles were not the target pest being studied, they were not counted and compared in this way. Though infestations visually appeared to be substantial in swaths of alyssum, fleabeetle damage did not affect final harvest of the Brussels sprouts that year at Woodman Horticultural Farm. From farm visits and discussions with local growers, we surmise that Woodman Horticultural Farm may have lower levels of fleabeetles than other farms in the Northeast (Sideman, personal communication). Alyssum hosting fleabeetles did not appear to have substantial deleterious effects on the cash

crop, however, farms with established historically high levels of fleabeetles should take this potential consequence into consideration.

Banker plants. Another biological control tactic used mainly by greenhouse growers is insectary plants that act as banker plants (Wäckers and van Rijn, 2012). These "banker" insectary plants host other insects that are not problematic pests to the cash crop but can augment or maintain populations of natural enemies (by acting as a habitat for their food source) during periods in which the target pest is not plentiful. This tactic may be worth perusing to augment or maintain hoverflies when target pests are scarce.

Figure 32. Fleabeetles and holes from fleabeetle damage on a Brussels sprout leaf in 2017.

Abundant hoverfly species

The most abundant hoverfly found throughout the season was *T. marginatus*, which represented 87.4% of all hoverfly specimen collected with the sweepnet. *T. marginatus* has also been cited as one of the top three hoverfly species collected from flowers in central Illinois over 33 years (Tooker et al., 2006). In the present study, *Syritta pipiens* and *Toxomerus politus* each represented 3.6% of all hoverfly specimen collected. Tooker et al. (2006) also reports *S. pipiens*

as one of the top three hoverfly species collected. However, *S. pipiens* is detritivorous (Tooker et al., 2006), which indicates that though it may have implications for pollination purposes, it is not likely to have much impact on aphid control. *T. politus* also may not be important for aphid control, as its larvae are phytophagous, feeding on pollen of corn (*Zea mays*) (Marin, 1969) and sorghum (*Sorghum bicolor*) in Brazil (Nunes-Silva et al., 2010). Therefore, *T. marginatus* was overwhelmingly the most abundant aphidophagous hoverfly in this study.

Conclusion

We observed alyssum to be a low maintenance, long-blooming insectary plant that hosts the most prevalent aphidophagous hoverfly species (*T. marginatus*) throughout the growing season. Buckwheat, cilantro, and dill hosted substantial hoverfly densities but had shorter bloom periods, requiring a creative solution to provide food resources throughout the season. Further research is needed to understand the specific relationship between regional hoverfly nutrition from insectary plants and their ability to control cabbage aphid in a field setting.

Some insectary plants attracted non-target insects and specifically to known *Brassica* pests. There were a significantly greater number of fleabeetles observed on alyssum in 2017 than all other insectary plant treatments. There were a significantly greater number of imported cabbageworm moths observed on calendula than all other insectary plants and hoverfly density was low on calendula compared to other treatments. All told, alyssum appears to be the most practical insectary plant to use in a simplified system with only one planting if fleabeetle infestations tend to not reach economic threshold in the cash crop.

CHAPTER 4:

RELATIONSHIP BETWEEN CABBAGE APHID POPULATIONS & NATURAL ENEMIES

INTRODUCTION

Growers worldwide have reported economically damaging populations of a pest called the cabbage aphid *Brevicoryne brassicae* (L.) on crops in the *Brassicaceae* (*Brassica)* family. There are several natural enemies that are known to cause deleterious effects on cabbage aphid. Since aphid colonies reproduce rapidly, an ideal biological control agent acts quickly while aphid populations are low (Zhang and Hassan, 2003). Many types of beneficial insects were observed in the agroecosystem in a preliminary experiment (Levy and Sideman, unpublished) but the current study narrows the focus to the most prevalent insects observed in our region that decrease cabbage aphid numbers. Insects discussed in the current study are: hoverflies (Diptera: Syrphidae : Syrphinae), cecidomyiids (Rondani) (Dipetera: cecidomyiidae: cecidomyiinae), and braconid wasps (Hymenoptera: Braconidae: Aphidiinae). Both the larval and adult life stages of the lady beetles eat aphids and presence of lady beetle eggs on *Brassica* crops are noted in the literature, however cabbage aphid are not a preferred food for lady beetles (Jankowska, 2005) and their ability to reduce cabbage aphid is thought to be insignificant (Gadomski et al., 1998).

An advantage of natural enemies is their ability to find aphids in areas below the leaf canopy that are difficult to reach effectively with foliar insecticides (Figure 33).

Figure 33. Insects on a Brussels sprout plant below the leaf canopy, 2017. The red circle in the upper left highlights cabbage aphid on a sprout bud. The blue circles on the lower left and upper right highlight hoverfly larvae at different ages, and the yellow circle highlights aphids that have been parasitized by braconid wasps.

Hoverfly larvae (Diptera: Syrphidae: Syrphinae). As discussed in Chapter 3, hoverfly larvae predate on aphids. The most abundant hoverfly species in sweepnet samples in the present study was ***Toxomerus marginatus***. Unlike other species documented in the literature, *T. marginatus* lay their egg(s) next to low populations of aphids, or even zero aphids (Bugg et al., 2008). There have been field studies (Hickman and Wratten, 1996) that showed predatory hoverflies can provide meaningful control of cabbage aphid. However, some studies showed limited control. Reasons for poor control appear to be a low overall hoverfly population or asynchrony between timing of cabbage aphid and predatory hoverflies (see detailed explanation in Chapter 1)(Ambrosino, 2006; Tenhumberg and Poehling, 1995).

Cecidomyiids (Dipetera: Cecidomyiidae: Cecidomyiinae). Cecidomyiids, like hoverflies, are from the order Diptera and the large family of *Cecidomyiidae*, which has more than 6,200 described species worldwide (Jaschhof et al., 2014). The adults, called midges, emerge from pupae in the soil (Harris, 1973; LeFevre and Adams, 1982). They are black, thin, delicate flies with bodies less than 5mm long (Jaschhof et al., 2014), characterized by their long, beaded antennae (Figure 34).

Within the family *Cecidomyiidae* is the subfamily *Cecidomyiinae* that is comprised of herbivores, fungivores, and predators (Jaschhof et al., 2014). We use of the term "cecidomyiids" in this chapter to describe the predacious *Cecidomyiinae*, specifically the aphidophagous species. Harris (1973) reported that there are at least five species of aphidophagous cecidomyiids described in the literature and six species that may sometimes feed on aphids, but taxonomic work remains. Harris (1973) concludes the most important aphidophagous cecidomyiids are from the genera *Aphidoletes* and *Monobremia*. A lot of effort is needed to identify species of *Cecidomyiinae*; it requires a cecidomyiid specialist who has information on immature stages,

adults of both sexes of the cecidomyiid and information on the host, since cecidomyiids can be highly host-specific (Jaschhof et al., 2014). Furthermore, all life stages are inconspicuous (Harris, 1973) and adult midges are active during the night time (Harris, 1973; Ruzicka and Havelka, 1998) which makes them difficult to capture for identification.

One aphidophagous cecidomyiid, *Aphidoletes aphidimyza* (Rondani), is the most abundant and widely distributed species of *Aphidoletes* (Dipetera: cecidomyiidae). It has been documented in Japan, Czechoslovakia, the former U.S.S.R, Austria, Germany, Finland, France, Netherlands, England, Wales, Italy, Israel, Egypt, Sudan, Canada, U.S.A, and specifically Hawaii (Harris, 1973). *A. aphidimyza* larvae are considerably smaller than hoverfly larvae and are a solid orange color, in contrast to the mottled pattern that is distinctive of hoverfly larvae.

Like the hoverfly adult, the cecidomyiid adult midge lays eggs on crop leaves near or within colonies of aphids that hatch into predatory larvae that eat aphids (Hafez, 1961; Jankowska, 2005; LeFevre and Adams, 1982). *A. aphidimyza* adults may lay eggs singly or in groups of up to 40 eggs that hatch into larvae within two to three days. Each female lives for approximately 14 days or less and lays approximately 100 eggs (Harris, 1973). The orange eggs (0.3 x 0.1mm) and larvae (2mm in length at maturity (LeFevre and Adams, 1982)), can be spotted with the naked eye during daylight hours among colonies of aphids (Harris, 1973), whereas the adult shelters itself under plant leaves during the day and becomes active for feeding and oviposition during the night or twilight (Harris, 1973; Ruzicka and Havelka, 1998). The larvae predate on aphids and after seven to 14 days (comprised of three larval instars) they drop from the crop leaves and burrow into the soil to pupate (Harris, 1973; LeFevre and Adams, 1982). The larvae create silken cocoons approximately a few millimeters below the soil surface

and adults emerge within one to three weeks. The entire lifecycle (Figure 35) can be completed in approximately three weeks in favorable conditions above 21°C (Harris, 1973).

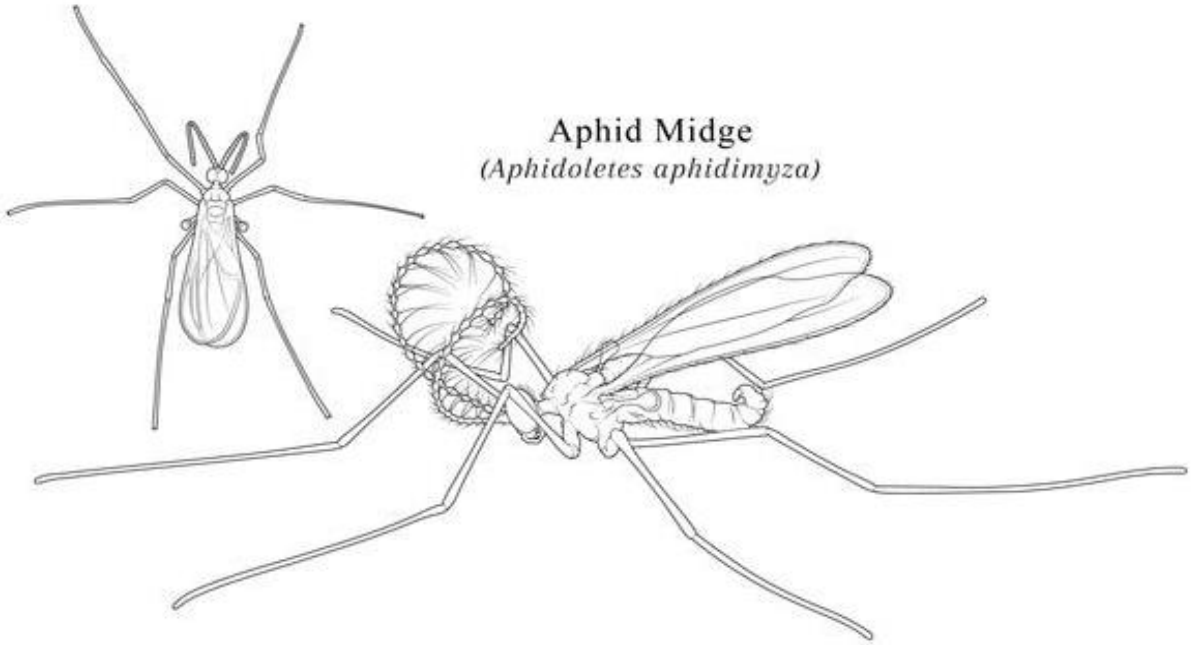


Figure 34. Female cecidomyiid midge (left) and male cecidomyiid male (right). Both male and female have characteristic long, beaded antennae, whereas a characteristic of the male is the hairs on their antennae. The life stage that is responsible for deleterious effects on aphids is the larva. ©Sound Horticulture, Illustration by Morgan Mahana, www.soundhorticulture.com. Reprinted with permission.

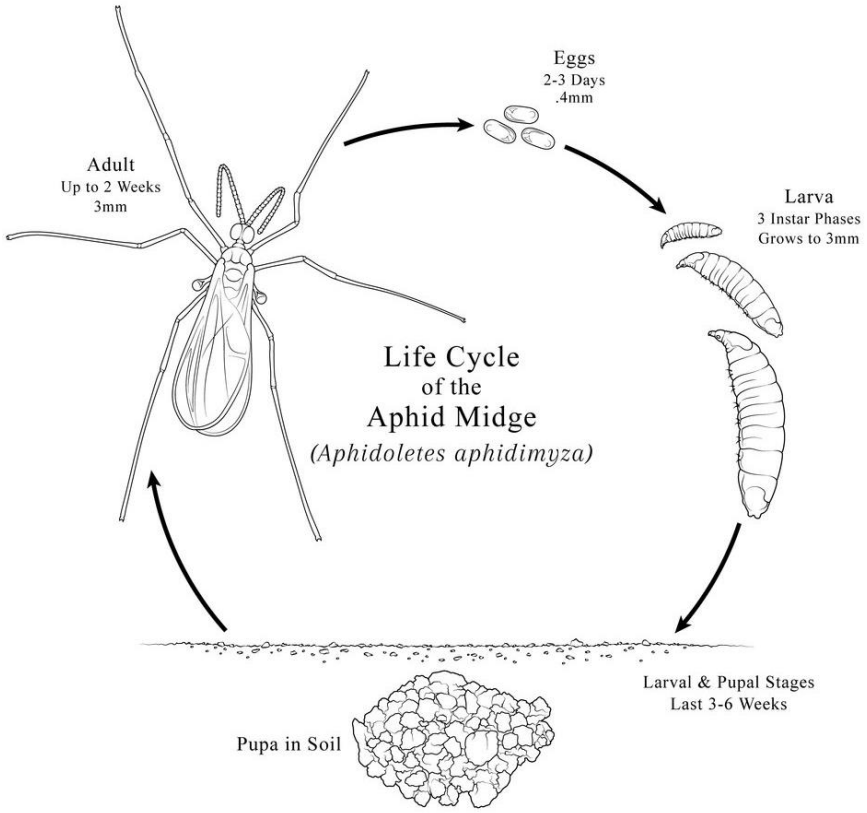


Figure 35. Lifecycle of cecidomyiids. The life stage that is responsible for deleterious effects on aphids is the hoverfly larva. Reprinted with permission from ©Sound Horticulture, Illustrations by Morgan Mahana, www.soundhorticulture.com.

A. aphidimyza larvae prey on aphids by biting their leg or other body joint and injecting a toxin that paralyzes the aphids (Harris, 1973; Jankowska, 2005). They subsequently suck out the inner fluids of the aphid body, leaving the skin behind (Harris, 1973; Jankowska, 2005; LeFevre and Adams, 1982). The deflated aphid bodies remain attached to the leaf by their stylet (piercing, sucking mouthpart) (Harris, 1973). Despite their small size, *A. aphidimyza* can eat aphids larger than themselves and when aphid populations are high, they may even kill aphids they do not eat (Harris, 1973; Jankowska, 2005).

Ruzicka and Havelka (1998) demonstrated that *A. aphidimyza* larvae can kill approximately 50-60 aphid adults (green peach aphid and *Aphis gossypii* (Glover)) during its larval stage, while George (1957) reported the destruction of 40-60 aphids during the larval

stage. According to Jankowska (2005) *A. aphidimyza* larvae may consume three to 50 cabbage aphids on a daily basis. Ruzicka and Havelka (1998) reported that larvae of *A. aphidimyza* are a promising biological control of aphids in an enclosed greenhouse setting. Jankowska, (2005) reported that *A. aphidimyza* have been successful in greenhouses on a commercial scale and advocate for the potential of its use as a biological control agent of cabbage aphid in the field.

Researchers have found mixed results in the response of *A. aphidimyza* to aphid number or aphid density. Ruzicka and Havelka (1998) demonstrated *A. aphidimyza* to be most effective on low populations of cabbage aphids. In one study *A. aphidimyza* showed an "inverse numerical response to prey density" (Hodek, 1966). They found than when prey density was high there were few *A. aphidimyza* found, but when prey density was low, many *A. aphidimyza* were found. Jankowska (2005) reported that *A. aphidimyza* may appear in numbers greater than 50 larvae per plant on plants with large colonies of aphids. In Lithuania, Duchovskienė et al. (2012) concluded that the number of naturally occurring *A. aphidimyza* in conjunction with parasitic wasp *D. rapae* were too few to provide sufficient reduction of cabbage aphid.

Pollard (1969) used methods that removed other predators (i.e. hoverfly larvae) from aphid-infested Brussels sprout leaves to evaluate the effect of cecidomyiids alone on cabbage aphid. First, they found that hoverfly larvae (*Syrphus balteatus* (Degeer) and *Sphaerophoria scripta* (L.)) were largely responsible for the suppression of cabbage aphid. However, they found that when hoverfly larvae were removed from Brussels sprout leaves, the number of cecidomyiid eggs that were laid were substantially greater (140) than ones that did not have hoverfly larvae removed (48). They observed a short delay before cecidomyiids (*Aphidoletes*) predation suppressed aphids entirely (Pollard, 1969). They conclude that without these predator

removal methods that cecidomyiid predation would not be possible to measure or appreciate (Pollard, 1969).

Timing of appearance of cecidomyiid adults is likely to affect the ability to control cabbage aphid. In Poland, Jankowska (2005) found that cabbage aphid were present in the field starting in the end of April, whereas the predatory larvae of *A. aphidimyza* were not observed until August. In England, Pollard (1969) observed cabbage aphid immigration into the field in July, whereas eggs of cecidomyiid (*Aphidoletes*) were observed on the second week of August. Literature is lacking on the occurrence of *A. aphidimyza* in the Northeast United States.

Braconid parasitoid wasps (Hymenoptera: Braconidae: Aphidiinae). The sub-family Aphidiidae (Hymenoptera: Braconidae) is a well-known aphid-attacking parasitoid wasp (Moayeri et al., 2013). We will use term "braconid" wasp to describe them if species is not specified. *Diaeretiella rapae* (M'Intosh) is a species that has been demonstrated to be the most important biological control of cabbage aphid (Bacci et al., 2009; Duchovskienė et al., 2010; Elliott et al., 1994; Jankowska and Wiech, 2003; Pike et al., 1999). *D. rapae* has been documented in Europe, U.S.A including Hawaii, Canada, and New Zealand (Hafez, 1961). The adult braconid wasp is a tiny insect, comparable to the size of a winged aphid (Harris 2017, personal observation) (Figure 36). The adult braconid wasp parasitizes the live aphid by depositing one or more eggs inside of the body of the aphid but only one of the young will survive to the adult stage (Hafez, 1961)(Figure 37). In 48 to 72 hours the wasp egg(s) hatch inside of the living aphid and the larva(e) feed on the inside of the aphid. The aphid may act sluggish but does not show obvious outward symptoms of parasitism until a few days later when the color changes to a light tan or golden brown (Hafez, 1961). At this point, the larva inside is at its full-grown stage and outwardly it is a hardened "mummified aphid." From the inside, the

larva will pierce through the outer layer of its aphid host and attach the mummy to the leaf using the threads from its cocoon (Hafez, 1961). A light nudge with the fingernail will show that the aphid is stationary and dead (Harris, personal observation). Subsequently, a single fully-formed adult braconid wasp will emerge from the aphid body (Hafez, 1961), which is then only a thin shell attached to the leaf with a hole where the wasp emerged ("hatched mummified aphid"). *D. rapae* can mate just a few minutes after emergence from its aphid host but the females do not require copulation to produce viable eggs. Virgin *D. rapae* can reproduce using arrhenotokous parthenogenesis, which allows unfertilized eggs give rise to all-male young (Hafez, 1961). They also can use sexual reproduction to fertilize eggs that give rise to both male and female progeny. Hafez (1961) found *D. rapae* to have five to 11 generations per year in the Netherlands. The shortest generation lasted approximately 15 days in the summer months and over colder months took up to 110 days to emerge from their host (Hafez, 1961). Dispersion of parasitoid wasps are considered to be mainly through their winged aphid hosts that have been parasitized and fly to a new location with the eggs or young stages in their body (Schlinger and Hall, 1960; Vevai, 1942).

Figure 36. Braconid wasp on a colony of cabbage aphids that appear both green and gray in color. There is a tan mummified aphid in the lower right. Photograph by Dr. Alan Eaton

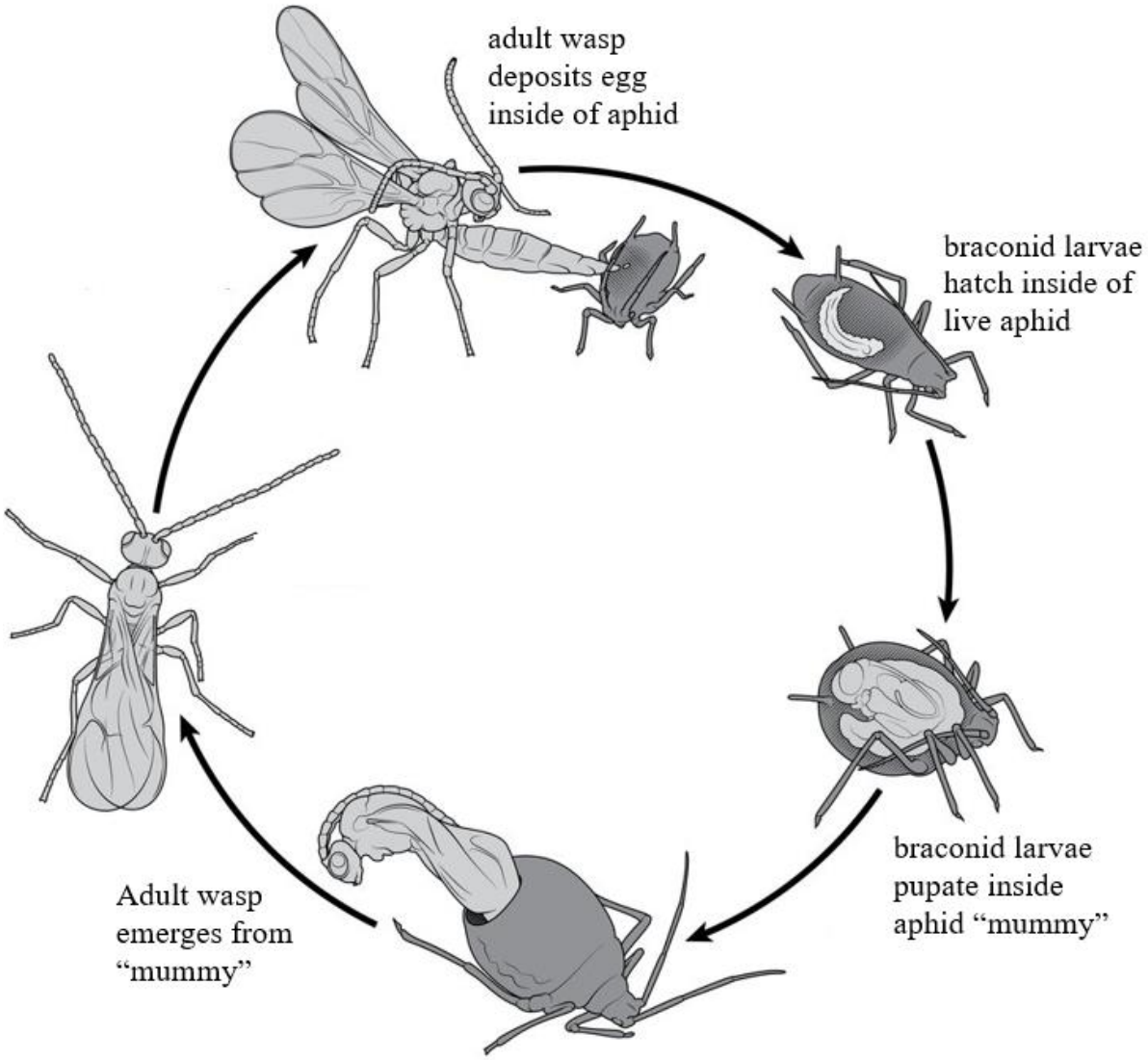


Figure 37. Lifecycle of a braconid wasp. ©Sound Horticulture, Illustrations by Morgan Mahana, www.soundhorticulture.com. Reprinted with permission. Diagram captions were changed from original diagram.

Like its aphid hosts, *D. rapae* is a cosmopolitan insect and has been known to parasitize over 60 aphid species (Rimaz and Valizadegan, 2013) including the Russian wheat aphid (*Diuraphis noxia* (Mordvilko)) (Reed et al., 1995) as well as aphids on *Brassica* crops (Desneux et al., 2005; Mussury and Fernandes, 2002; Stary, 1975). However, only five or six aphid species are preferred by *D. rapae* (Baer et al., 2004; Bayhan et al., 2007), which include cabbage

aphid (Baer et al., 2004; Hafez, 1961; Khakasa et al., 2016; Stark and Acheampong, 2007). *Myzus persicae* (green peach aphid) is a common pest of *Brassica* crops and may attack crops with cabbage aphid simultaneously. It has been demonstrated that *D. rapae* prefers to parasitize cabbage aphid over the green peach aphid (Hafez, 1961). Furthermore, Hafez (1961) in Netherlands and Sedlag (1958a) in Germany states that *D. rapae* was likely the sole parasite of cabbage aphid. In some published notes by Sedlag (1958b) they state that 99% of primary parasites emerging from the cabbage aphid were *D. rapae* and Gabrys (2008) states that *D. rapae* is the only primary parasitoid of cabbage aphid.

Not all attempts of parasitism by *D. rapae* are successful, but it has been demonstrated that adults produce over forty cabbage aphid mummies in their lifetime and will parasitize all instars of cabbage aphid without preference (Zhang & Hassan, 2003). Zhang & Hassan (2003) also demonstrated that parasitized cabbage aphids in the third instar, fourth instar, and adult life stage will still produce progeny, but substantially fewer (less than half) than their non-parasitized counterparts. They also demonstrated that parasitized cabbage aphid in the first and second instars (four days or younger) did not produce any progeny. Host plant water status may also influence parasitism rates. Tariq et al. (2013) demonstrated that brassica plants under drought stress had significantly fewer cabbage aphids parasitized by *D. rapae* and that *D. rapae* preferred the odor of plants that had not undergone drought stress.

Sedlag (1958a) discusses that although *D. rapae* parasitism of cabbage aphid is substantial, the levels of hyperparasitism (namely *Charipinae*) may affect their ability to control cabbage aphid. He concluded that *D. rapae* was not likely to be an adequate biological control agent of cabbage aphid, specifically due to the lack of aphid hosts when overwintering *D. rapae*

emerge early in the season. Gabrys, (2008) reports that *D. rapae* may reduce cabbage aphid populations by 30-40% at peak infestation.

Inundative release of natural enemies. One biological control strategy is the inundative release of natural enemies into the environment to assure that pest arrival and parasitoid presence coincides. Purchased beneficial insects are commonly released in commercial greenhouse settings (Jankowska, 2005) where the beneficial insects are confined to the pest area and high value cash crops can offset the expense. Commercially available natural enemies of aphids include a cecidomyiid (*A. aphidimyza*), hoverflies, (*E. balteatus*, and *Sphaerophoria ruppellii* Wiedemann) and a braconid wasp (*D. rapae*) (Emden & Harrington, 2007; Neuville et al., 2016; van Lenteren et al., 2018).

Researchers in France studied cabbage aphid on cabbages in the field and tested the effect of different release times of braconid wasps (*D. rapae*) on aphid parasitism (Neuville et al., 2016). They used a ratio of one parasitoid female per five cabbage aphids. *D. rapae* were released at the beginning of aphid infestation, seven days after infestation, and 14 days after infestation. They found that *D. rapae* released at the beginning of aphid infestation resulted in 89.6% parasitism a month later (Neuville et al., 2016). In contrast, they found that the same rate of parasitoids released a week after cabbage infestation resulted in 63% parasitism a month later, and *D. rapae* released two weeks after initial aphid infestation resulted in only 39.7% parasitism. Researchers concluded that introduction of braconid wasps at the time of cabbage infestation maximized interactions between the pest and parasitoid early in the growing season (Neuville et al., 2016).

Zhang & Hassan (2003) evaluated successive release of *D. rapae* into cages that contained potted cabbage plants with cabbage aphids. They used a ratio of six female wasps to

five aphid adults (six times more wasps than Neuville et al. (2016)) and released *D. rapae* on the first, third, and sixth day of the experiment, which resulted in an 88.9% aphid parasitism rate by the 20th day of the experiment. These methods that used the successive release of *D. rapae* onto cabbage plants with aphids resulted in a continuous supply of *D. rapae* wasps that emerged from parasitized aphids for a two-week period. In another experiment they then transplanted cabbage plants infested with both young cabbage aphids and mummies parasitized by *D. rapae* into broccoli field plots to compare against broccoli plots that did not have infested cabbage plants nearby (Zhang & Hassan, 2003). Seven weeks after infested cabbage plants were transplanted into the field, their results showed that the infested cabbage plant treatment increased aphid parasitism but did not control cabbage aphid. They found that 93.3% of broccoli plants in the treated plots contained parasitized aphids compared to control plots where 56.7% of broccoli plants contained parasitized aphids. However, an economically meaningful control of cabbage aphid was not obtained in either treatment; as only 6.7% and 1.4% of aphids were parasitized on infested cabbage plots and control plots, respectively. Zhang & Hassan (2003) concluded that more releases of *D. rapae* were necessary for control of the pest in a field setting. However, purchasing natural enemies for inundative releases is expensive and may not be an economically feasible tactic to control cabbage aphid in a field setting. The present study focuses on conservation augmentation of natural enemies in the agroecosystem by providing a hospitable environment.

The effects of flower nutrition on parasitoid wasps have also been investigated. A greenhouse study of *D. rapae* by Araj and Wratten (2015) evaluated the impact of flowering species on survival (days), number of mature eggs, and number of parasitized cabbage aphids. Araj and Wratten (2015) evaluated alyssum (cv. Carpet of Snow), buckwheat (cv. Katowase),

and two common weeds (white rocket and shepherd's purse) against a water control with no flowering plants. They demonstrated that mean survival (number of days lived) was significantly greater in buckwheat (9.14) than alyssum (5.43). Provision of alyssum had a significantly greater mean survival compared with the water control (2.29) and shepherd's purse (4.14) but was not significantly different than white rocket (4.43).

Araj and Wratten (2015) also evaluated the mean number of mature eggs that *D. rapae* laid over 48 hours with provision of the same treatments. Provision of buckwheat resulted in the highest number of mature eggs per female (383.8). Buckwheat was not significantly different than alyssum (350.6) and both resulted in significantly greater number of mature eggs per female *D. rapae* than the water control (233.2). Alyssum was not significantly different than the flowering weed species.

In addition, Araj and Wratten (2015) evaluated the mean number of parasitized aphids per *D. rapae* female and found that wasps on a buckwheat diet parasitized significantly more cabbage aphids (233.0) than all other treatments. Alyssum (186.1) was not significantly different than shepherd's purse (175.3) or white rocket (173), however, all flowering treatments resulted in significantly greater number of parasitized aphids per female. The results of Araj and Wratten (2015) demonstrate that provision of alyssum in the field may not increase the effectiveness of *D. rapae* compared with common weeds. However, since weeds are commonly killed prior to bloom in crop fields, provision of alyssum may increase the effectiveness of *D. rapae*.

Preliminary results from 2016 that included insectary flowering plants in the same field as Brussels sprouts in New Hampshire (Levy and Sideman, unpublished) reported cabbage aphid parasitism from braconid wasps. Despite the notable rates of parasitism on Brussels sprouts, there was not an economically relevant decrease in aphid population correlated with insectary

plants, such as that provided by foliar spray organic-approved insecticides. We collaborated with researchers in Massachusetts that performed a very similar experiment to the methods described in chapter 3 and evaluated insect populations on insectary plants using sweepnets. They captured substantial numbers of *D. rapae* and hyperparasitoids (*Alloxysta* sp.) (Scheufele and Higgins, unpublished). The present study does not focus on the effects of hyperparasitoids on parasitoids of aphids, but it is important to note their existence.

Flower nutrition to braconid wasps. The results of Araj and Wratten (2015) demonstrate that provision of alyssum in the field may not increase the effectiveness of *D. rapae* compared with common weeds. However, since weeds are commonly killed prior to bloom in crop fields, provision of alyssum may increase the effectiveness of *D. rapae*. Furthermore Araj and Wratten (2015) concluded that nutrition from buckwheat provided significantly greater number of days lived and mean number of mature eggs than alyssum.

Effect of distance from floral resources on hoverfly occurrence. Lovei et al. (1994 and 1997) report equal populations of hoverflies in crop fields for 20-30 meters away from insectary plants, after which the populations of adult hoverflies decrease. Hickman et al. (1995) studied the pollen found in the gut of hoverflies in relation to pollen resources available and demonstrated that hoverflies utilize crop and insectary plant pollen as a food source. For example, they confirmed large quantities of cilantro in the digestive tract of gravid females (Hickman et al., 1995). We would like to know if insectary plants can be used to enhance natural predation and parasitism of cabbage aphid. We would also like to know if there is a decline in predation or parasitism in greater proximity to insectary plants to determine how best to deploy insectary plantings as part of an integrated control strategy. This information will help

us evaluate the cost/benefit of including insectary habitat plants in *Brassica* crop agroecosystems.

Goals, objectives, hypotheses

The overall goal of this study was to investigate which natural insect predators and parasitoids suppress cabbage aphid. The three specific objectives of this experiment were to: (1) Identify natural enemies of cabbage aphid present in a Brussels sprout agroecosystem; (2) Quantify the populations of natural enemies of cabbage aphid on Brussels sprout leaves over time; and (3) Test the effects of distance from insectary plants on insect predation and parasitism of cabbage aphid. We hypothesized that natural enemies of cabbage aphid would be present, and that predation and parasitism of cabbage aphid would be highest near insectary plants.

MATERIALS AND METHODS

Experimental design. Approximately 1224 square feet were planted with a continuous swath of alyssum (insect refuge) that directly abutted a field of 9,520 square feet of Brussels sprout plants planted at 18-inch spacing between plants, formatted in 12 rows. Three different experimental designs were used to meet the objectives of this chapter.

First, to quantify the natural enemies of cabbage aphid on Brussels sprout leaves, we used the experimental design from chapter 2 that evaluated insecticide treatments, but for this experiment we solely observed plants in the control plots that were only sprayed for lepidopteran pests. In both years there were three replications, and plots were comprised of three rows of Brussels sprout plants. In 2017 each plot was comprised of 36 plants and in 2018 plots were comprised of 30 plants. In both years, nine plants (three plants from each row) per plot were chosen at random for counting insects on their leaves. For each plant, two leaves each from the lower, middle, and upper portion of the plant were observed for insect count on both sides of the leaves, six leaves total per plant. The purpose of these data were to simply quantify population dynamics on control treatment leaves over time, and therefore data were not analyzed for statistical differences.

Second, to test the effects of distance from insectary plants on predation and parasitism of cabbage aphid **on Brussels sprout leaves,** we used rows that had only been sprayed for lepidopteran pests that ran the entire length of the field, ranging from zero to 107 feet from the insectary plantings. Each of the three rows was a replication; each containing five plots with varying proximity to the insectary habitat plant. Each plot consisted of 6 plants, planted in one row (Figure 38). Six leaves were sampled as described in the previous paragraph.

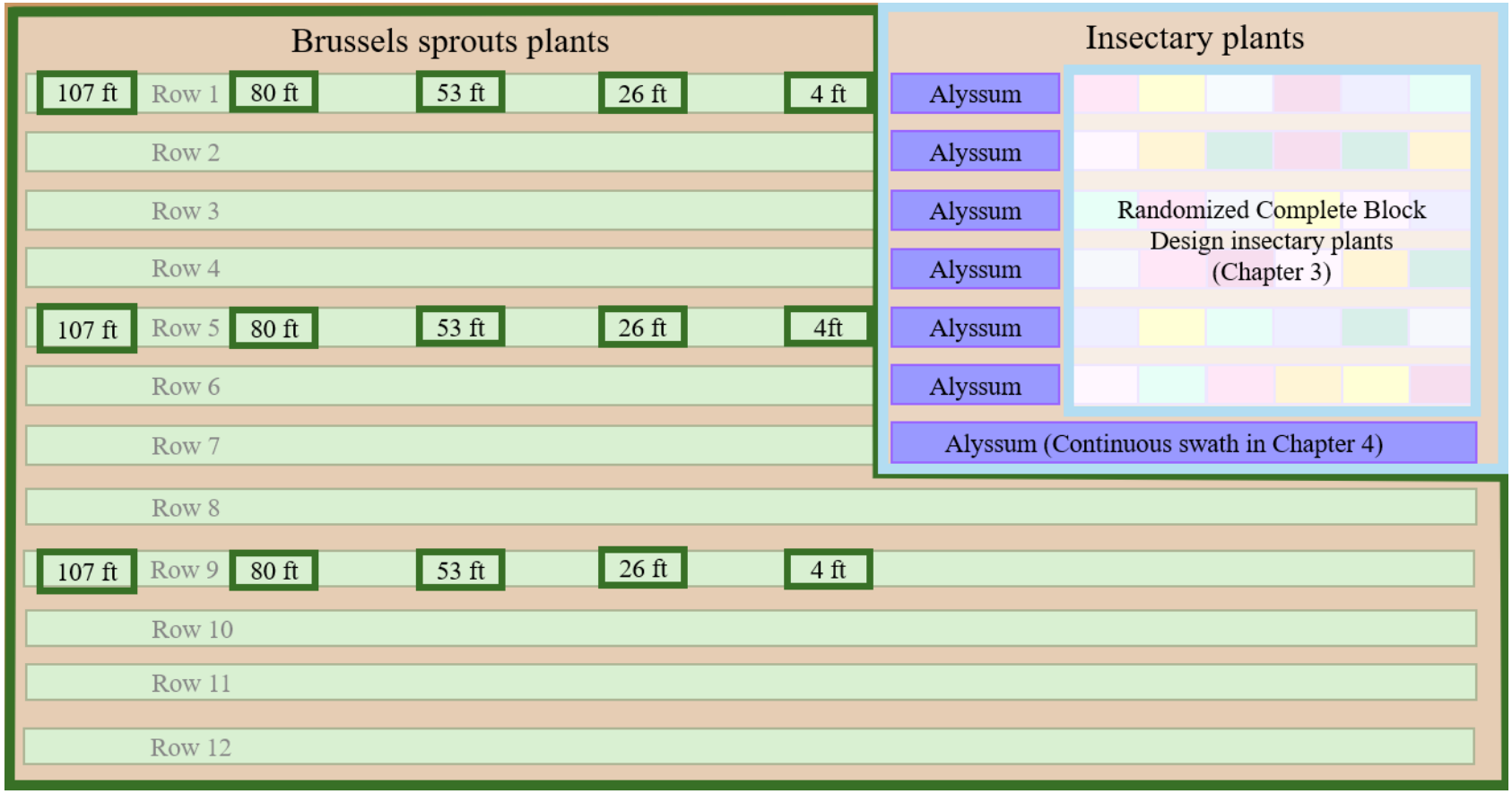


Figure 38. Field layout of Brussels sprout plants and insectary plants. This layout was used test the effects of distance from insectary plants on predation and parasitism of cabbage aphid. Distance treatments ranged from 4 ft to 107 ft and had three replications. The bold green boxes labeled with distances represent plots of six Brussels sprout plants in a single row. Figure is not drawn to scale.

Third, to test the effects of distance from insectary plants on distribution of parasitoids and predators of cabbage aphid using **sticky traps**, we used rows that had only been sprayed for lepidopteran pests as transects and varying distances were the treatments. We evaluated the number of parasitoids and predators at five sites ranging from a mean proximity of four feet away from insectary plants up to 107 feet away. The five treatments were replicated three times in rows (Figure 38). Sticky traps were placed under severely infested Brussels sprouts plants, leaned against the base of the Brussel sprout stalk and stuck to the black plastic mulch between the plants.

Seedling production. We selected alyssum (*Lobularia maritima*) for our insectary plant species because it has been shown to attract natural enemies of aphids in other studies (Brennan, 2013; Colley and Luna, 2000; Hogg et al., 2011) and its indeterminate flowering habit provides a continuous bloom period. 'Sweet' alyssum and 'Diablo' Brussels sprout (Johnny's Selected Seeds, Albion, Maine) seeds were sown into 128-cell trays on 24 May 2018 and 31 May 2018 using Promix BX (Pro-Mix, Quakertown, PA) soilless media and grown in a greenhouse until transplant at Woodman Farm in Durham, NH. All seedling trays were fertilized with water soluble fertilizer two times before transplant [15N–2.2P–12.5K] (Peters Professional 15–5–15 Cal–Mag; Everris Intl., Geldermalsen, The Netherlands) at a rate of 300 ppm N.

Field site preparation. Field experiments were conducted in the same field in 2017 and 2018 at the University of New Hampshire at Woodman Horticultural Farm in Durham, New Hampshire, United States. Prior to transplanting and direct seeding into the field, the entire field had 150 lbs/acre of nitrogen (N) as 27-0-0 incorporated in the spring based on soil test recommendations. Soil where Brussel sprouts were to be planted also received 50 lbs/acre of potash (K_2O) as 0-0-60. Six-foot spaced beds were created with 1 line of drip tape buried 1 inch

below the soil surface. Raised beds were covered with 0.6 mil Organix A.G. Film biodegradable black plastic mulch (Organix Solutions, Phoenix, Arizona) and slits were cut into the plastic mulch to seed and transplant insectary plant treatments. Alyssum seedlings were transplanted at 20 days after seeding on 11 Jul 2017 and 20 Jun 2018 at about 4-inch spacing. Details of Brussels sprout transplanting, spacing, insecticide applications, topping, and irrigation can be found in Chapter 2.

Data

Number of natural enemies of cabbage aphid on Brussels sprouts leaves over time. Signs of parasitism and predation were observed on infested Brussels sprout plants over 11 sample dates throughout the season. It is not realistic to observe significant numbers of braconid wasps in the act of parasitizing to measure the rate of parasitism. Instead we counted signs (or indicators) of parasitism. We counted the number of parasitized aphids ("mummies") and parasitized winged aphids ("winged mummies") to see if braconid wasps favored parasitizing aphids with or without wings. We counted hatched parasitized aphids ("hatched mummies") to confirm that the progeny was viable and successfully emerged as adult wasps. Additionally, braconid wasp adults were counted if they were found on a leaf. We also counted the following predators: cecidomyiid larvae, hoverfly larvae, brown lacewing (Neuroptera: Hemerobiidae), adult lady beetles, and lady beetle larvae.

In 2018, cabbage aphid numbers were so few that the present study could not be replicated. Therefore, only 2017 data are presented.

Effects of distance from insectary plants on predation and parasitism of cabbage aphid on Brussels sprout leaves. Signs of parasitism and predation were observed on infested Brussels sprout plants that did not receive any spray treatments for cabbage aphid on 10 Oct 2017 at

varying mean distances (four, 26, 53, and 80 feet) from insectary plants. Predators and signs of parasitism were recorded as described in the previous paragraph. We counted number of adult braconid wasps, non-winged mummies, winged mummies, hatched mummies (sum of winged and hatched mummies), non-winged aphids, winged aphids, cecidomyiid larvae, and hoverfly larvae were recorded.

Effects of distance from insectary plants on insect predation and parasitism of cabbage aphid on sticky cards. Sticky trap data were collected only once, during the end of the growing season when untreated plants were fully infested. Yellow sticky cards with adhesive on both sides were deployed on 10 Oct 2017 by leaning them against the base of Brussels sprouts plants below the leaf canopy and collected 20 Oct 2017.

Once sticky cards were collected, predators and parasitoids were counted on both sides of the card using a 10x hand lens under the supervision of Dr. Alan Eaton. Each side of the sticky card was visually divided in thirds to enable counting precision of many small insects. We counted number of adult braconid wasps, cecidomyiid midges, brown lacewings, hoverfly larvae, hoverfly adults, larvae of multicolored Asian lady beetle (*Harmonia axyridis*) and "other parasitica." The group called "other parasitica" was comprised of mostly chalcids (*Hymenoptera: Chalcidoidea*), a super family of parasitoid insects. However, it is not likely that these "other parasitica" were responsible for parasitizing cabbage aphid and were likely seeking a different insect to parasitize (Eaton, personal communication). Therefore, "other parasitica" numbers were reported separately. These "other parasitica" appear very similar braconid wasps to the naked eye, but differences were distinguished by the length of their antennae.

Statistical analysis

All experimental designs had three replications and insects on Brussels sprout leaves were counted on six leaves per plant and on both sides of each leaf. To report the number of natural enemies on Brussels sprout leaves over time, insects were summed for the six leaves per plant, and data from nine plants in each plot were used to calculate means per six leaves for each observation date. To report the effect of distance from insectary plants on predation and parasitism, insects were summed per six leaves or per sticky trap. This count data did not have normal distribution and did not respond to transformation. With JMP Pro statistical software, we ran a General Log-Linear Model, assuming a Poisson distribution. We then conducted a contrast test for means separation at $\alpha=0.05$.

RESULTS

Number of natural enemies of cabbage aphid on Brussels sprouts leaves over time.

Winged cabbage aphids were observed in plots on Brussels sprout leaves, starting 26 Jul 2017, which was also the first day that cecidomyiid larvae were observed (Figure 39 and Figure 46). Non-winged progeny of cabbage aphid appeared by 1 Aug 2017 (Figure 45). Mummified aphids (Figure 40, Figure 41 and Figure 44) were found even before the first winged cabbage aphids. These early mummies were likely from another species of aphid (Green peach aphid) found early in the season on the seedlings. The first braconid wasps (Figure 40) were found on 9 September, and almost three weeks later the first hatched mummified aphids (from which braconid wasps emerged) (Figure 40 and Figure 44) were observed on 28 Sep 2017, which also coincided with the first day that hoverfly larvae (Figure 41) were observed on Brussels sprout leaves (Figure 46). The numbers of cabbage aphid and cecidomyiid larvae continued to rise and were greatest on 28 Sep 2017, after which populations began to subside on 17 Oct 2017 (Figure 46). On the same date, number of hoverfly larvae peaked while braconid wasp numbers continued to rise, peaking at the last sample date on 2 Nov 2017 (Figure 46). Aphid predators such as brown lacewings (Neuroptera: Hemerobiidae), adult lady beetles, and multicolored Asian lady beetle larvae (Figure 43) were observed and counted, however numbers were low and are not presented.

In 2018 there were low levels of cabbage aphid, and in turn, low levels of predators and parasitoids. Therefore, natural enemy numbers are not presented, but we were surprised to observe a tiny spider hauling off a winged aphid in 2018 (Figure 42).

Figure 39. Orange cecidomyiid larva next to two cabbage aphids on a leaf. Photograph is approximately to scale.

Figure 40. Adult braconid wasp on a mummified aphid attached to a Brussels sprout leaf.

Figure 41. Brussels sprout leaf infested with cabbage aphid. Three hoverfly larvae are highlighted with blue circles. Note that the different colors of hoverflies likely show that the larvae belong to different species of hoverfly. An adult braconid wasp is highlighted with a pink circle in the upper right, and there are many tan mummified aphids, some of which are highlighted with yellow circles. Photo is approximately to scale.

Figure 42. A tiny spider hauling off a winged cabbage aphid on a Brussels sprout leaf in 2018.

Figure 43. Braconid wasp and multicolored Asian lady beetle larva on a Brussels sprout leaf stem under the leaf canopy. Photograph is approximately to scale.

Figure 44. Mummies (parasitized aphids) still attached to Brussels sprout leaf. Many have a hole where the parasitoid wasp emerged.

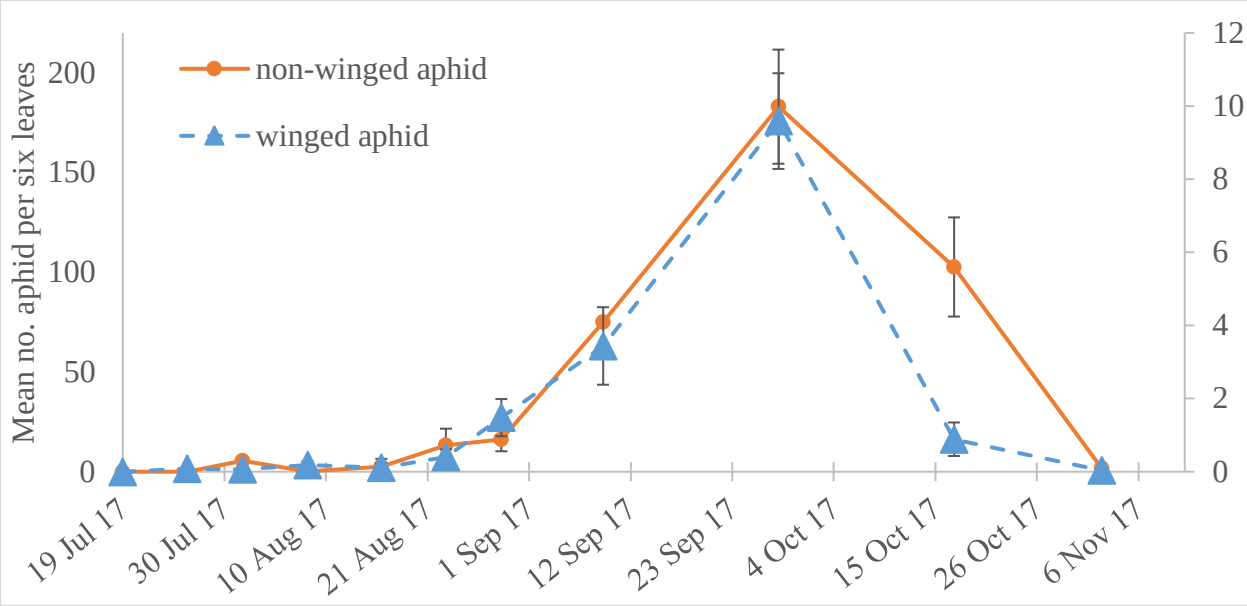


Figure 45. Mean number (± SEM) of non-winged cabbage aphid and winged cabbage aphid per six leaves on control plots over 11 sample dates from 19 Jul 2017 to 2 Nov 2017. The left axis is for non-winged cabbage aphid data, which are plotted with solid lines and circular markers. The right axis is for winged aphid data, which are plotted with dotted lines and triangular markers.

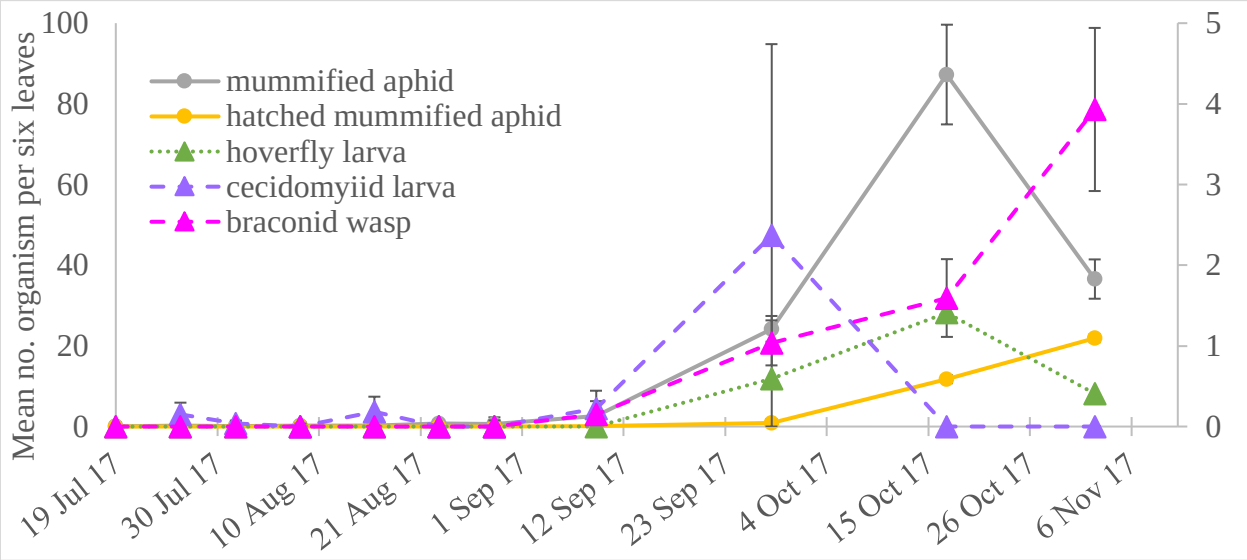


Figure 46. Mean number (± SEM) of natural enemies per six leaves on control plots over 11 sample dates from 19 Jul 2017 to 2 Nov 2017. The left axis includes mummified aphids and hatched mummified aphid data which are plotted with solid lines and circular markers. The right axis includes hoverfly larva, cecidomyiid larva, and adult braconid wasp data, which are plotted with dotted lines and triangular markers.

Confounding variables. There were a few confounding variables that could have contributed towards inaccuracies. Plants growing in the east side of the field, which was furthest away from the insectary plants, were stunted and appeared nutrient deficient. It is possible that the end of the field had lower applications of Nitrogen because of a fertilizing error when the field was prepared in spring 2017. Because of this the plots the furthest away from insectary plants (107 feet) were omitted from analyses.

Effects of distance from insectary plants on predation and parasitism of cabbage aphid. Our results did not clearly show that aphid parasitism or presence of predators and parasitoids increased with proximity to insectary plants. Significant differences in numbers of aphids, predators, and parasitoids over distance were observed in many cases, however, the distances with the highest levels of beneficial insects varied. There were significant differences in number of non-winged aphids in all distance treatments, which ranged from 258.4±44.8 at 26 feet to 368.3±35.2 at 80 feet (Table 11). There were significantly fewer non-winged mummies at distances of 4 feet and 26 feet than distances of 53 and 80 feet and ranged from 83.9±12.2 at 4 feet to 119.9±10.6 at 80 feet (Table 11). The number of hatched mummies (sum of winged hatched mummies and non-winged hatched mummies) also were significantly fewer at 3 feet and 26 feet than distances of 53 and 80 feet, ranging from 7.8±1.62 at 4 feet from insectary plants to 13.1±2.34 at 80 feet (Table 11).

The number of winged aphids followed a different pattern. Mean distance of 4 feet from insectary plants had had a significantly greater number of winged aphids than mean distance of 80 feet. Winged aphids ranged from 5.0±1.22 at 80 feet to 12.7±2.52 at 4 feet (Table 11). The number of winged mummies followed a similar pattern and ranged from 0.83±0.23 at 80 feet to 2.0±0.78 at 4 feet (Table 11).

Adult braconid wasps were significantly fewer at a mean of 26 feet away from insectary plants, compared to other distance treatments (Table 11). There were no significant differences between leaf observations at the different distances for both cecidomyiid and hoverfly larvae and numbers were low (Table 11). The frequency of parasitism (ratio mummies to aphids) ranged from 0.267±0.018 to 0.368±0.043 but had no significant differences in distances ($\boldsymbol{p}$=0.9431, χ^2 = 0.385, df = 3, 6).

*Table 11. Mean number (± SEM) of insects per six Brussels sprout leaves at increasing distances from insectary plants on 10 Oct 2017. Six leaves per plant were summed and means were calculated from three replicates. For all columns, df= 3,6. The **p**-value is from the Chi-squared test.*

Distance from insectary plants	Braconid wasp	Non-winged mummy	Winged mummy	Hatched mummy[z]	Non-winged aphid	Winged aphid	Cecido-myiid larva	Hoverfly larva
4 feet	4.2±0.7 b[y]	83.9±12 a	2.0±0.8 b	7.8±1.6 a	304.3±28 b	12.7±2.5 c	0.72±0.4	2.8±0.6
26 feet	2.6±0.7 a	86.4±24 a	1.7±0.4 b	8.2±2.7 a	258.4±45 a	6.6±1.3 ab	0.72±0.4	2.3±0.8
53 feet	4.2±0.8 b	116.7±11 b	1.3±0.5 ab	12.1±3.2 b	349.8±36 c	6.4±1.3 ab	1.00±0.7	2.9±0.6
80 feet	3.7±0.6 b	119.9±11 b	0.8±0.2 a	13.1±2.3 b	368.3±35 d	5.0±1.2 a	0.94±0.5	3.7±0.6
p	**0.0379**	**<0.0001**	**0.0171**	**<0.0001**	**<0.0001**	**<0.0001**	**0.7146**	**0.1337**
χ^2	8.433	196.9	10.18	38.31	415.8	75.13	1.361	5.584

[z] Hatched mummy is the sum of winged hatched mummies and non-winged hatched mummies.

[y]Within column, insect means followed by the same letter are not significantly different according to contrast tests, following a General Log-Linear Model test, assuming a poisson distribution at $\alpha = 0.05$.

Effects of distance from insectary plants on predators and parasitoids on sticky cards.

Sticky cards did not show that presence of predators and parasitoids increased with increased proximity to insectary plants. The number of braconid wasps was significantly greater at 107 feet than 4 feet from insectary plants and ranged from 76.3±38.4 to 121.3±50.4 respectively. Number of other parasitica ranged from 15.0±1.15 at 80 to 30.0±12.7 at 107 feet. Mean distance of 80 feet had significantly fewer number of other parasitica than distances of 4 feet and 107 feet. Cecidomyiid midges (Figure 47), brown lacewings, hoverfly larvae, hoverfly adults, and lady beetle larvae had low numbers and there were no significant differences between distance treatments (Table 12).

Figure 47. A cecidomyiid midge on a sticky trap with characteristic beaded antennae, likely an Aphidoletes aphidimyza male. The larval stage of this insect predates on cabbage aphid. The sticky trap was deployed below infested Brussels sprout plants on 10 Oct 2017 and collected on 20 Oct 2017. Photo credit and identification: Dr. Alan Eaton, former Cooperative Extension Integrated Pest Management specialist.

*Table 12. Mean number (± SEM) of insects per yellow sticky card at increasing distances from insectary plants. Means were calculated from 3 replicates of sticky cards that were deployed at the base of infested Brussels sprout plants on 10 Oct 2017 and collected on 20 Oct 2017. For all columns, df= 4,8. The **p**-value is from the Chi-squared test.*

Distance from insectary plants	Braconid wasp	Other parasitica	Cecidomyiid midge	Brown lacewing	Hoverfly larva	Hoverfly adult	Lady beetle larva
4 feet	76.3±38.4 a[z]	28.7±20.2 c	0.67±0.7	0.33±0.3	1.0±0.6	0.7±0.3	0.3±0.3
26 feet	89.7±26.2 ab	20.4±12.8 ab	0.33±0.3	0±0	0.3±0.3	0±0	0±0
53 feet	87.7±24.8 ab	18.3±2.96 ab	0.67±0.3	0±0	0.7±0.3	0±0	0.3±0.3
80 feet	86.3±21.7 ab	15.0±1.2 a	0±0	0±0	0±0	0±0	0±0
107 feet	121.3±50.4 c	30.0±12.7 c	0±0	0±0	0±0	0±0	0±0
p	**<.0001**	**0.0001**	**0.2358**	**0.5219**	**0.1269**	**0.1688**	**0.4532**
χ^2	35.84	23.07	5.545	3.219	7.176	6.438	3.665

[z]Within column, insect means followed by the same letter are not significantly different according to contrast tests, following a General Log-Linear Model test assuming a poisson distribution at $\alpha = 0.05$.

DISCUSSION

Cabbage aphid parasitoid and predators were observed within and near aphid colonies throughout the season and appear to contribute to cabbage aphid suppression. Mummies and larvae were counted over time, but without a control plot with methods of insect removal like Pollard (1969), it is difficult to quantify the effect of the natural enemies on number of aphids. We did, however, quantify that aphid populations still occurred at levels above the economic threshold and natural insect enemies did not provide control of the pest in 2017. After severe infestations, aphid numbers crashed likely due to the epizootic of entomopathogenic fungi.

Synchronization of the biological control with the pest. Synchronization of the pest and biological control is key for efficient pest suppression (Neuville et al., 2016). Timing of hoverfly and cecidomyiid larvae arrival differed from results that have been published elsewhere. In Poland, Jankowska (2005) found that hoverfly larvae appeared about 3 months after cabbage aphid, whereas in the present study they appeared just over one month after cabbage aphid. Similar to our observations, Pollard (1969) in England observed cabbage aphid immigration into the field in July, and hoverfly larvae (*Syrphus balteatus* (Degeer) and *Sphaerophoria scripta* (L.)) were observed in early August. For cecidomyiid predatory larvae, Jankowska (2005) showed that they arrived 3 months after cabbage aphid, Pollard (1969) found that they arrived a few weeks after cabbage aphid, and we found that they were present at the very beginning of the study, even before cabbage aphid were detected.

Zhang and Hassan (2003) described the release of reared *D. rapae* into the field by transplanting aphid and mummy infested cabbages in between broccoli plants. Their methods used *D. rapae* with cabbage aphid as the host, which introduced beneficial *D. rapae*, but also introduced more cabbage aphid. We hypothesize that equal or greater cabbage aphid suppression

could be obtained by using an alternative host plant (not *Brassica*) infested with aphids that are host-specific (and that do not target *Brassicas*). Current commercial greenhouse methods use purchased *D. rapae* as biological control for aphids and when aphids are sparse on the cash crop, growers provide *D. rapae* with "banker plants" which harbor aphids that are host-specific and not pests to the cash crop. *D. rapae* has been known to parasitize other aphid species, including the Russian wheat aphid (*Diuraphis noxia* (Mordvilko)) (Reed et al., 1995), whose host is wheat (not a *Brassica*) and could potentially be a good insectary banker plant to evaluate for suppression of cabbage aphid. Since inundative release of natural insect enemies are likely to be cost prohibitive in an outdoor setting, we propose melding the methods of Zhang and Hassan (2003) with banker plants. For example, transplanting wheat plants that are infested with parasitized and healthy Russian wheat aphids into *Brassica* crop fields to build up parasitoid populations prior to when winged aphids distribute themselves into crop fields in the summer. If the Russian wheat aphid is not a pest to the surrounding crops, *D. rapae* population may be augmented prior to winged aphid distribution into the crop and without potentially increasing aphid pest pressure on the cash crop. This method may also result in augmented synchronization of the pest and its natural enemy.

Effects of distance from insectary plants on predators and parasitoids. Since flowering plants have been known to increase natural enemy insect fitness (Amorós-Jiménez, Pineda, Fereres, and Marcos-García, 2014) and in turn suppression of aphids, we hypothesized that a closer proximity to insectary plants would increase the rate of parasitism and number of predators, however, the data collected did not support this hypothesis.

The number of non-winged aphids, which account for most of the aphid population were significantly greater on Brussels sprouts leaves that were farthest away from the insectary plants

compared to all other treatments. Plants on the east side (furthest from insectary plants) appeared nutrient deficient, and despite omitting the fifth distance treatment from this analysis it is likely that this possible nutrient inconsistency affected the number of cabbage aphid distribution in the field. As there were significantly fewer non-winged aphids on Brussels sprout leaves in the distance treatment closest to the insectary plants, it follows that braconid wasps, non-winged mummies, and hatched mummies were also fewer. The results from sticky traps showed similar results, in that number of braconid wasps also were fewer on treatments with closer proximity to the insectary plants. Between observations on Brussels sprout leaves and sticky traps, number of cecidomyiid midges, cecidomyiid larvae, brown lacewings adults, hoverfly adults, hoverfly larvae and lady beetle larvae were low and did not have significant differences between distance treatments. In 2018 there were low levels of cabbage aphid, and in turn, low levels of predators and parasitoids prevented meaningful replication and comparison of aphid predation and parasitism in 2017.

In the present study, we compared distances ranging from four feet to 80 feet on Brussels sprout leaves as well as four feet to 107 feet using sticky traps. Lovei et al. (1994 and 1997) reported populations of hoverfly larvae decreasing in distances further than 20-30 meters (60-90 feet) from insectary plants. Hickman and Wratten (1996) conclude that the beneficial effect of insectary plants (phacelia) on insects (hoverflies) continued for 180 meters (540 feet) from the insectary planting. The distances evaluated in the present study were much shorter than that of Hickman and Wratten (1996) and similar to Lovei et al. (1994 and 1997); future experiments that aim to test the effects of distance on natural enemies should include longer distances from insectary plantings to observe if there is a "cut off" on the distance that parasitoids and predators are willing to fly from insectary plants. It would be prudent for future researchers to replicate

this type of experiment with prevailing wind coming from the opposite direction to determine the effects of wind on dispersal.

Laubertie (2007) points out an important aspect of the relationship of flowers to hoverfly predation rates. Though the provision of flowering plants attract and feed hoverflies required nutrients for reproduction, it cannot be assumed that eggs will be laid near insectary plants (Laubertie, 2007). White et al. (1995) found that although there were more adult hoverflies caught in traps with closest proximity to a phacelia border, the number of hoverfly eggs on the crop did not differ between treatments with and without phacelia borders. Hoverfly, braconid wasps and cecidomyiid adults have wings and are highly mobile insects that have been demonstrated to fly long distances (Laubertie, 2007). Chandler (1968) demonstrated there was no difference in hoverfly oviposition within small plots of Brussels sprouts that included provision of flowers in buckets compared with plots without provision of flowers.

Conclusion

Cabbage aphid parasitoids, parasitized aphids, and predators were observed within and near aphid colonies throughout the season. Hoverflies, cecidomyiids and parasitic braconid wasps appear to contribute to cabbage aphid suppression. However, cabbage aphid populations still occurred at levels above the economic threshold in 2017 and natural insect enemies did not provide full control of the pest. Our results suggest that regional growers who use these growing methods and implementation of insectary plants may provide partial suppression of cabbage aphid, are unlikely to achieve full control. Other methods for the control of cabbage aphid should be considered to use in conjunction improve control of cabbage aphid but minimize harmful effects to the beneficial fungi and natural insect enemies.

It remains unclear if proximity to insectary plants affects the rate of predation and parasitism of cabbage aphid. We propose future experiments to test the effects of distance on natural enemies to include greater distances from insectary plantings to observe if there is a "cut off" distance that these highly mobile parasitoids and predators are willing to fly from insectary plants. Furthermore, attention to nutrients and water consistency throughout the field is also key for accurate results.

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